1

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In this issue
Rob Siebers, Editor

Serum prostate specific antigen (PSA) and free PSA are widely used as conventional serum markers for prostate cancer diagnosis. Metastasis-associated lung adenocarcinoma transcript 1 (MALAT-1) is a type lncRNAs and recent studies have highlighted it as a promising biomarker in prostate cancer diagnosis and prognosis. Festus Idomeh and colleagues evaluated the diagnostic utility of total PSA, free PSA, percentage free/total PSA ratio, histology biopsy and expression pattern of MALAT-1 in prostate cancer patients in Southern Nigeria. They found that that MALAT-1 gene is a better biomarker for prostate cancer risk, diagnosis and prognosis.

Measuring total serum bilirubin is considered the gold standard for neonatal jaundice but requires a blood sample. New, non-invasive devices allow for screening of healthy term neonates via transcutaneous bilirubin measurement. Melanie Adriaansen and colleagues assessed the accuracy of the Philips Bilichek® and Scanmedics Bilicare™ transcutaneous bilirubin devices against laboratory total serum bilirubin measurements in a New Zealand population. They found that overall, the Bilichek® had a slightly higher correlation and lower variability than the Bilicare™. The Bilichek® was also more user-friendly and was their device of choice.

Dennis Mok and colleagues present a management review input checklist for ISO 15189:2012 internal auditing as an optimisation guide for medical laboratories in this issue.

The presence of numerous antibiotic-resistance mechanisms in Gram-positive and negative bacteria is a global concern, which is further complicated by emergence of newer mechanisms in recent years. Few new compounds are in the production-pipeline that show potential for usage as antimicrobial agents. Antibiotic adjuvant ceftriaxone-disodium edetate-sulbactam, available under tradename Elores™, showed potential in a study by Mohd. Shahid and colleagues from India demonstrating antimicrobial activity against various multidrug-resistant bacteria.

Vancomycin-resistant-Enterococci (VRE) has become a challenging nosocomial pathogen. Infections with VRE can pose therapeutic problems and are associated with increased patient morbidity and mortality worldwide. Hiba Sami and colleagues conducted a study to find out the prevalence of linezolid resistance, VRE and *vanA* gene in enterococcal isolates in a tertiary care hospital of North India. They found an alarming emergence of linezolid resistance. Furthermore, the presence of VRE and *vanA* gene in significant numbers in a clinical setting is also worrying and indicates a need to implement strict antibiotic stewardship programme to prevent the spread of resistant isolates and losing all treatment options.

Karrar Algershi and colleagues from Iraq evaluated a new POCT haemoglobinometer (URIT-12) against a cell counter method. They found 48.6% false elevated haemoglobin levels and conclude that the URIT-12 is not suitable as a POCT device in evaluating haemoglobin levels in anaemic patients.

Acute appendicitis is the most important cause of referral of patients with acute abdominal pain to the emergency department. Procalcitonin has emerged as a promising biomarker for acute appendicitis and in this issue Ali Khavanin and colleagues from Iran evaluated the diagnostic accuracy of serum procalcitonin in the diagnosis of acute appendicitis.They found that although procalcitonin seems to be a high-sensitivity test for the diagnosis of acute appendicitis, because of its low specificity, the use of other criteria, especially % polymorphonuclear neutrophils, seems to be necessary in this regard.

Norovirus is recognised to be a major cause of gastroenteritis among children worldwide. Favour Osazuwa and colleagues conducted a study to determine the prevalence and genetic diversity of norovirus among children under 5 years with diarrhoea in South–South, Nigeria. Norovirus was detected in 11.1% of children with diarrhoea. Genogroup II (GII) norovirus was detected in 84.4% of patients, while genogroup I (GI) norovirus was identified in 15.6% of patients. Genotype diversity was large as demonstrated by nine identified genotypes.

Vitamin B_{12} (Vit B_{12}) and folate are essential micronutrients for early neural development in the fetus. Jinny Ng and colleagues investigated Vit B_{12}, folate and Hb levels in neonates born at Middlemore Hospital and determined if a difference exists in these micronutrients in the ethnic groups in the area. They found that Vit B_{12} and folate levels at birth are similar to levels seen in adults at Middlemore Hospital. Results from the various ethnic groups included in the study shows some variation in Vit B_{12}, folate and Hb levels. Also, an elevation in cord blood Hb was associated with elevated Vit B_{12} levels.

Refeeding syndrome is a poorly recognised and understood condition with no universally accepted definition. It occurs after unbalanced re-introduction of caloric intake following prolonged starvation and has distinct metabolic disturbances. In this issue Natasha Dutt and Samarina Musaad from Auckland describes a case of hyponatremia and hypochloraemia caused by refeeding syndrome. It is characterised by deranged phosphate, potassium, magnesium and water balance within the body. Awareness of refeeding syndrome, its risk factors and biochemical findings are important to enable timely management.

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Diagnostic utility of total PSA, free PSA, percentage PSA, histology biopsy and expression pattern of MALAT-1 in prostate cancer patients in Southern Nigeria

Festus Aigbokheo Idomeh, Humphery Benedo Osadolor and Olalekan Olusola Elekofehinti

ABSTRACT

Objective: This study examines the diagnostic utility of serum levels of total, free and percentage prostate specific antigen (PSA), histological biopsies and metastasis-associated lung adenocarcinoma transcript 1 gene (MALAT-1).

Methods: Fifty-two patients were recruited for this study from the urology clinics of a tertiary and private based Hospital in Benin City. Serum samples obtained from participants were used to assay for total and free PSA by enzyme linked immunosorbent assay (ELISA). The expression pattern of MALAT-1 was detected using reverse transcriptase polymerase chain reaction (RT-PCR). Histological biopsy was done for 39 participants whose PSA levels were greater than 10ng/ml. A one-way ANOVA post hoc multiple comparisons and Student's t-test were used for comparison of groups while receivers operating characteristics (ROC) curve were used to determine their diagnostic utility.

Results: There was a significant up regulation of MALAT-1 gene in all prostate cancer patients and some benign prostatic hyperplasia (BPH) patients compared to controls (P<0.001). Biopsies revealed 26 of the participants had prostate cancer while 13 were diagnosed with benign prostatic hyperplasia. There were significant variations in prostate cancer risk biomarkers: total PSA ng/ml (P<0.05), free PSA ng/ml (P<0.05) and percentage PSA % (P<0.05) in prostate cancer patients compared to BPH and control participants. The diagnostic performance of using area under the ROC curve (AUROC) revealed MALAT-1 gene had the highest AUROC of 0.964.

Conclusion: This study revealed that MALAT-1 gene is a better biomarker for prostate cancer risk, diagnosis and prognosis.

Key words: Prostate cancer, PSA, MALAT-1 gene, reverse transcriptase polymerase chain reaction.

INTRODUCTION

Prostate cancer is the second leading cause of cancer related deaths worldwide (1). It has been reported that prostate cancer has complex, multi-factorial etiology, genetic and environmental factors which contribute to its development (2). The level of awareness of prostate cancer is poor in Nigeria with a prevalence of between 2 % and 11 % (3-5).

Serum prostate specific antigen (PSA) is widely used as a conventional serum marker for prostate cancer diagnosis and can predict cancer risk and treatment outcome (6,7). In some clinical settings, free PSA other than total PSA is used in the diagnosis of prostate cancer. Though most clinical decisions are still based on total PSA, there is still more compelling reasons to base it on % free PSA. Percentage free PSA appears to be most clinically useful when PSA reaches levels of 4 to 10ng/mL, hence estimating it's ratio can improve the specificity in monitoring prostate cancer and decrease the number of negative biopsies in patients (8). When total PSA is in the range of 4.0-10.0 ng/mL, a percentage free:total PSA ratio is useful in the differential diagnosis of BPH and prostate cancer. Depending on the ratio, the probability can be determined as follows (9).

% f/t PSA ratio	Probability of prostate cancer
0-10	55%
10-15	28%
15-20	25%
>20	10%

Though PSA testing has positively influenced the diagnosis and treatment of prostate cancer, it's limitations has resulted in large numbers of unnecessary biopsies and overtreatment (10,11). Hence, the development of new biomarkers is urgently needed for clinical prostate cancer screening (12).

Long noncoding RNA (lncRNA) is an essential population of the noncoding RNAs (ncRNA) with a sequence of over 200 nucleotides (13). They are classified as tumour suppressor genes or oncogenes according to their specific function and expression pattern in tumour tissues (14). Metastasis-associated lung adenocarcinoma transcript 1 (MALAT-1) is a type lncRNAs and recent studies have highlighted it as a promising biomarker in prostate cancer diagnosis and prognosis (15).

This study therefore evaluated the diagnostic utility of total PSA, free PSA, percentage free/total PSA ratio, histology biopsy and expression pattern of MALAT-1 in prostate cancer patients in Southern Nigeria.

MATERIALS AND METHODS

Subjects
This study was carried out at University of Benin Teaching Hospital and Faith Mediplex Hospital urology outpatient clinics. Twenty-six confirmed cases of males with prostate cancer, thirteen confirmed benign cases and thirteen apparently healthy age matched control participants with an average age of between 40-79 years old.

Ethics
Ethical approval was obtained from the State Ministry of Health, Edo State, Nigeria (HM.1208.297).

Inclusion and exclusion criteria

Patients with clinical evidence of prostate cancer and BPH with no other histories of reproductive and endocrine system disorders, underlying disease or other forms of cancer that consented were recruited for this study.

Study design

The participants were grouped into A, B and C.
- Group A: Prostate cancer participants (positive rectal examination, PSA level of >4ng/ml and a positive prostate biopsy).
- Group B: Benign prostatic hyperplasia (positive rectal examination, PSA level >4ng/ml and a negative prostate biopsy).
- Group C (negative rectal examination, PSA level <4ng/ml).

Blood sample collection

Venous blood samples were obtained from each participant before rectal examination and dispensed into an RNA shield and plain tube. The blood containing RNA shield tube was mixed and stored at -20 ºC until analysis. The other blood sample in a plain tube was allowed to clot, the serum retracted and stored in another anticoagulant-free specimen bottle. The serum samples were stored at 2-8 ºC for two months before analysis.

Total PSA assay

Total PSA was assayed by Accu-Bind ELISA (Monobind Inc, Lake Forest, USA) (16).

Free PSA Assay: Free PSA was assayed by Accu-Bind Enzyme linked immunosorbent Assay Microwell Method by Monobind Inc, Lake forest, USA (16),

Percentage free PSA

Percentage PSA was measured as the percentage of free (uncomplexed) PSA relative to the total amount of PSA in men (7).

Biopsy histology

A trans-rectal (12 core) biopsy was obtained by a physician guided by a trans-rectal ultrasound through the anus into the rectum. Biopsies were then processed and stained using the Haematoxylin and Eosin (H&E) staining technique and the tissue samples examined under the microscope to determine the Gleason score (17).

Polymerase chain reaction methods (Total RNA extraction using the Zymo Research whole-blood RNA MiniPrep)

Total RNA was extracted using the Zymo Research whole–blood RNA MiniPrep according to the manufacturer's specificcations (Zymo Research, Irvine, CA, USA). The targeted genes were then visualized by PeQlab UV transilluminator and photographed. Molecular weights were calculated using molecular weight standard of the marker. The gene expressions were displayed on bar charts (18).

RESULTS

Table 1 shows that total, free and percentage PSA were significantly higher in prostate cancer participants compared to others (P<0.05). Figure 1 indicates the ISUP grades of positive prostate biopsies obtained in this investigation.

The Gleason scores and International Society of Urological Pathological Grades of histological biopsies result from prostate cancer participants is illustrated in Figure 2.

The diagnostic performance of serum levels of total, free and % PSA, MALAT-1 gene and histological biopsies (Figure 3) was determined using area under the ROC curve (AUROC). The MALAT-1 gene has its assay conditions illustrated in Table 2 and had the highest AUROC of 0.964 followed by histological biopsies of the prostate tissues which had an AUROC of 0.879, free PSA 0.829, percentage PSA 0.800 and Total PSA 0.786. The expression patterns of MALAT-1 gene are illustrated in Figures 4 to 7 and the overall expression of MALT-1 gene from all participants in this study is shown in Figure 8.

Table 1. Serum levels of total, free and percentage PSA in all participants. Results are mean ±SEM.

Biomarkers	Prostate cancer (n=26)	BPH (n=13)	Control (n=13)	F	BP
Total PSA	55.24 ± 1.34	19.18 ± 1.02	1.44 ± 0.11	455.41	0.002
Free PSA	13.05 ± 0.21	4.99 ± 0.23	0.82 ± 0.05	198.22	0.003
% PSA	8.13± 0.27	19.01 ± 0.27	38.12 ± 1.25	125.11	0.004
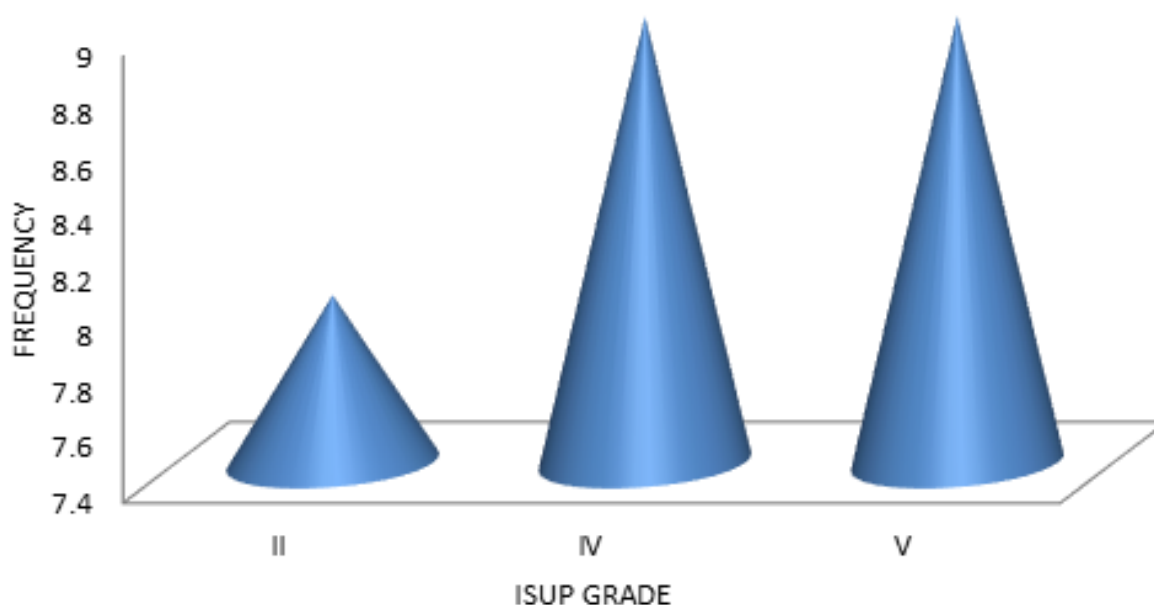
Figure 1: Grades of positive biopsies of prostate cancer participants

Table 2. Assay condition for MALAT-1 gene evaluated and purchased from Inqaba Biotechnology Industries, Hartfield, South Africa.

Gene symbol	Primer sequence	Molecular weight	GC%	Temperature (min/max)	Length	Ascession number
MALAT-1 Forward	GGGTGTTTACGTAGACCAGAACC	7088.67	52.17	57.06	23	GSE33455
MALAT-1 Reverse	CTTCCAAAAGCCTTCTGCCTTAG	6934.56	47.83	55.25	23	

A
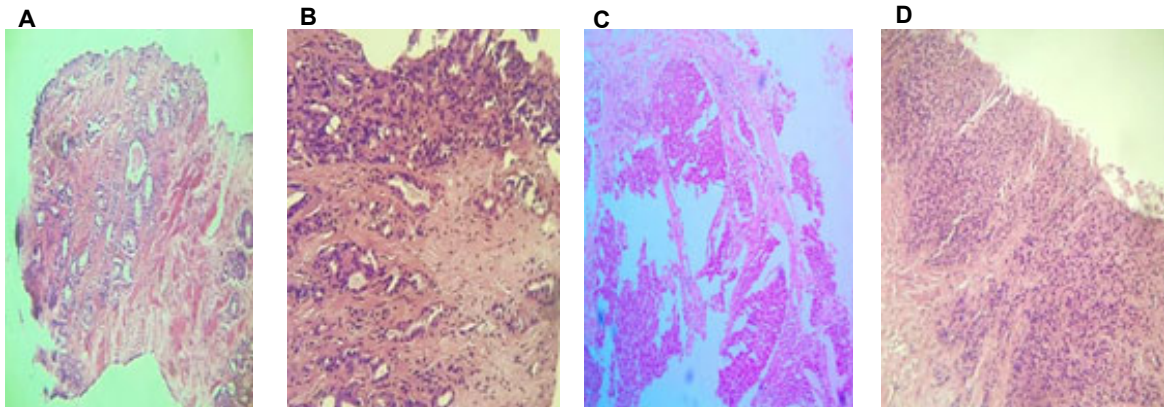
Gleason score 2: A circumscribed nodule containing small glands with some variations in shape, separated by stromal tissue in amounts less than one gland's width.

B

Gleason score 3: Single, separate, infiltrative, much more variably sized glands, closely packed or irregularly separated. Some of the larger glands are angulated. There are small glands which have pin-point lumina and are crowded but still separated by stroma.

C

Gleason score 4: Small, fused (cribriform), infiltrative glands. There is no intervening stroma between neighbouring glands.

D

Gleason score 5: Single cells haphazardly arranged or forming short cords without glandular lumina. This pattern may be mistaken for lymphocytic infiltrates at low magnification.

Figure 2. Different histological appearances of the various Gleason patterns of prostate cancer as viewed under the microscope (Haematoxylin and eosin x100).
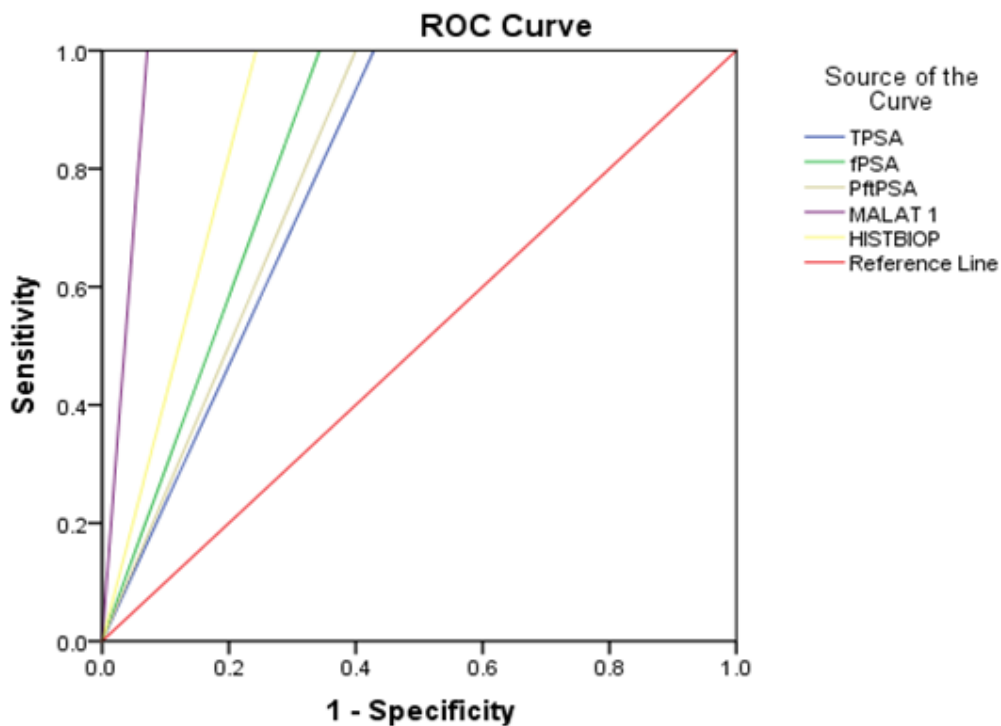
Figure 3. Diagnostic performance of serum levels of total, free and % PSA, MALAT-1 gene and Histological biopsies
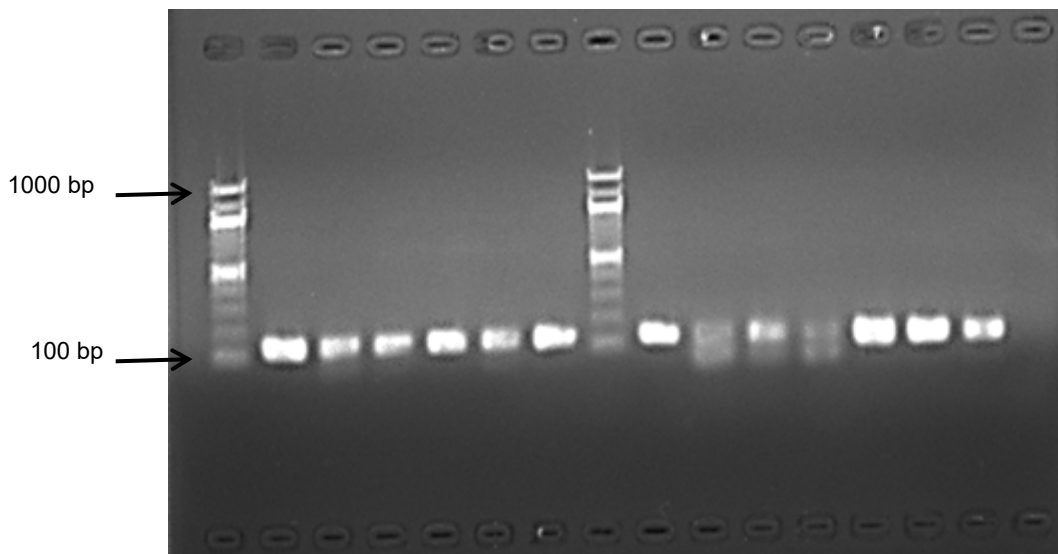
Figure 4. Reverse transcriptase polymerase chain reaction (RT-PCR) results for MALAT 1 gene analysed on a 1.0 % agarose gel electrophoresis stained with ethidium bromide. L is 100-1000 bp DNA ladder. A represents prostate cancer participants. Samples 1A, 2A, 3A, 4A, 5A, 6A, 11A, 12A, and 13A are positive for MALAT-1 gene with bands at 100 bp. Samples 8A, 9A and 10A are positive for MALAT-1 gene with bands at 95 bp and 100 bp. Sample NC is a no template control.
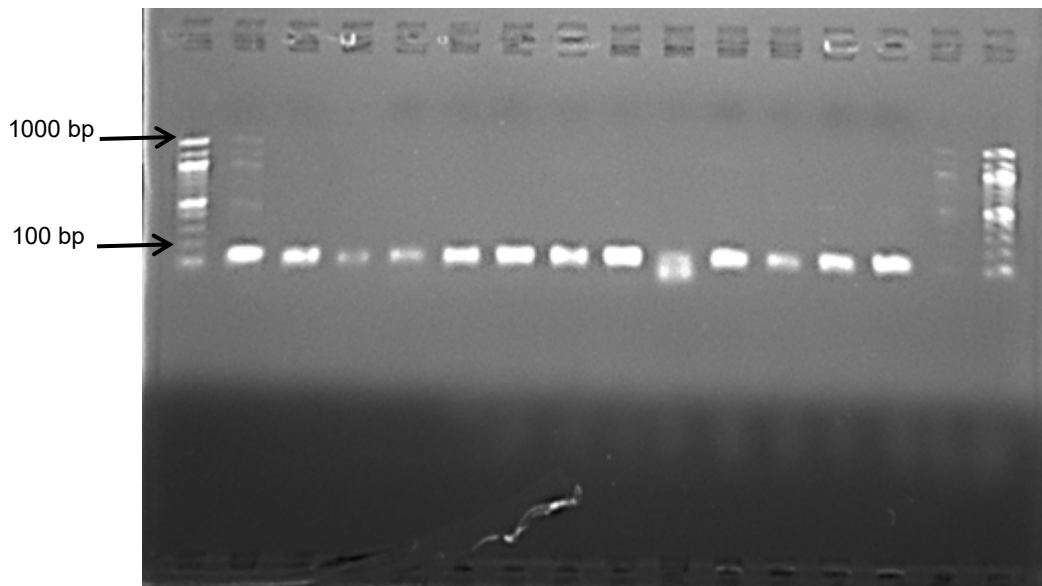
Figure 5. RT-PCR results for MALAT-1 gene analysed on a 1.0 % agarose gel electrophoresis stained with ethidium bromide. L is 100-1000 bp DNA ladder. A represents prostate cancer participants. Samples 14A, 15A, 16A, 17A, 18A, 19A, 20A, 21A, 23A, 24A, 25A and 26A are positive for MALAT-1 gene with bands at 100 bp. Sample 22A is positive for MALAT-1 gene with bands at 95 bp and 100 bp. Sample NC is a no template control.
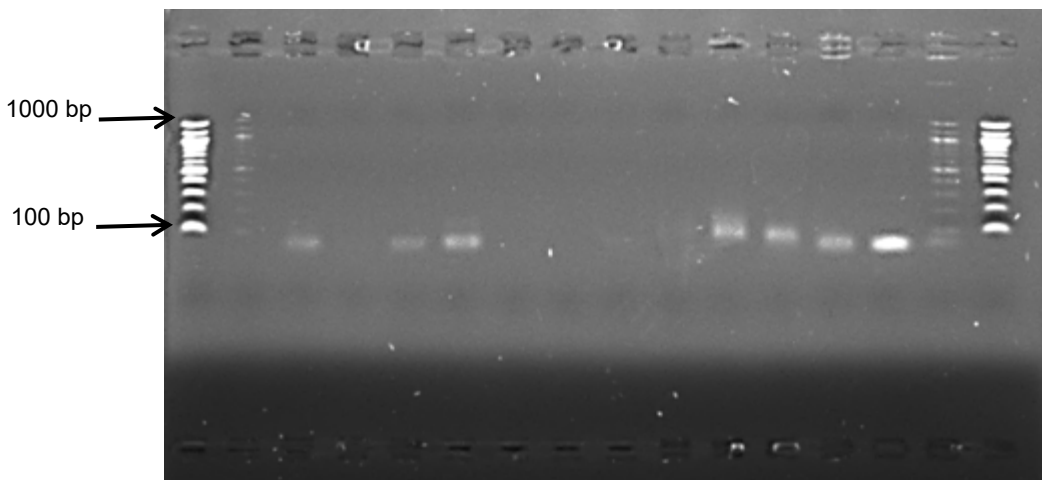
Figure 6. RT-PCR results for MALAT 1 gene analysed on a 1.0 % agarose gel electrophoresis stained with ethidium bromide. L is 100-1000 bp DNA ladder. B represents benign prostatic hyperplasia participants. Samples 2B, 4B, 5B, 9B, 10B, 11B and 12B are positive for MALAT-1 gene with bands at 95 bp. Samples 1B,3B, 6B, 7B, 8B and 13B are negative for MALAT-1 gene. Sample NC is a no template control.
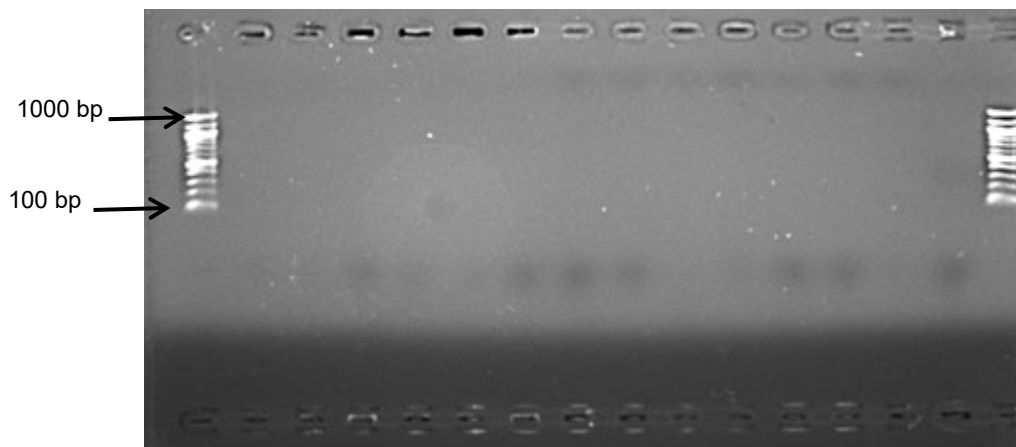
Figure 7. RT-PCR results for MALAT 1 gene analysed on a 1.0 % agarose gel electrophoresis stained with ethidium bromide. L is 100-1000 bp DNA ladder. C represents the control participants. All samples are negative for the MALAT-1 gene. Sample NC is a no template control.
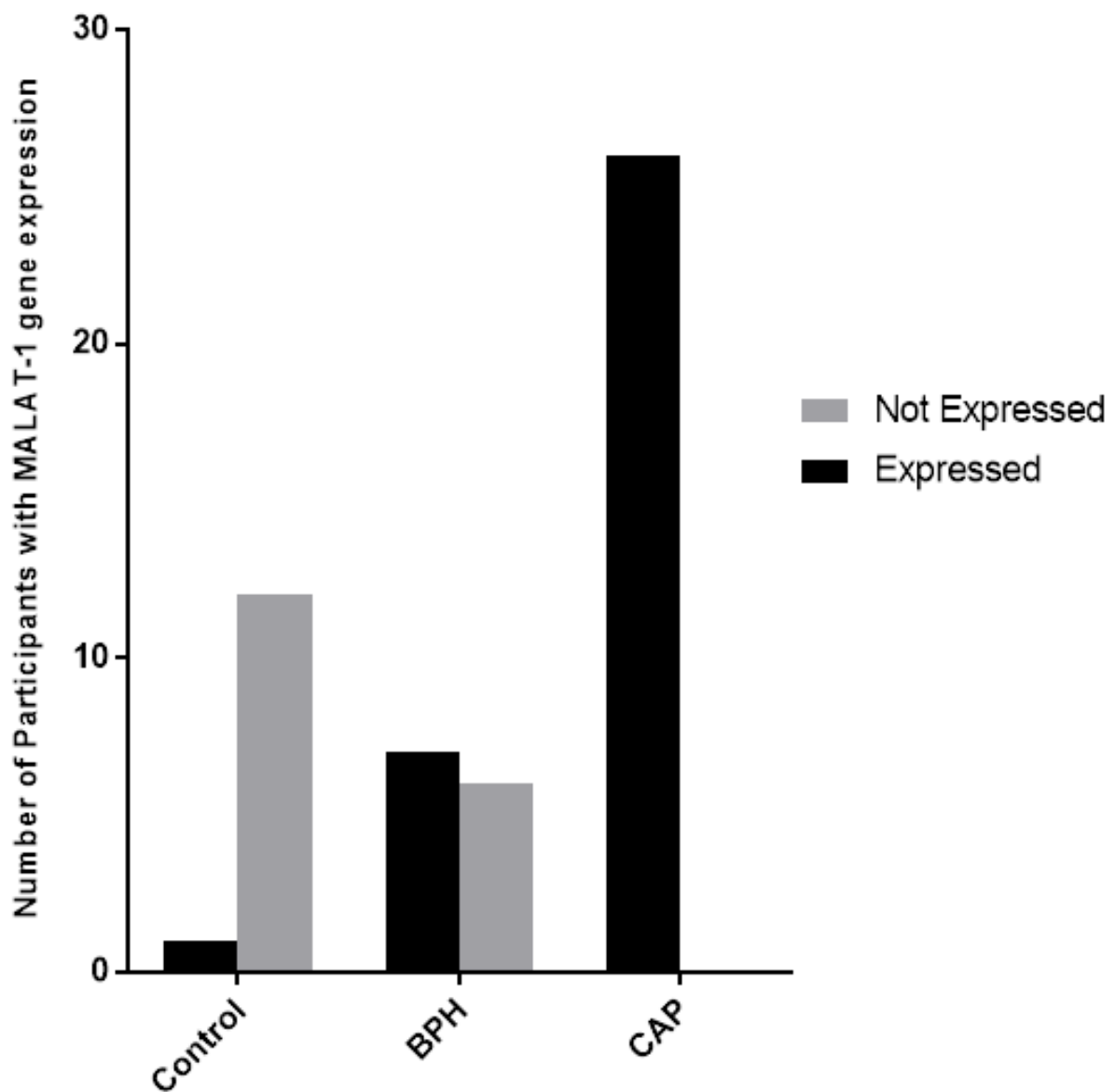
Figure 8. An overall multiple bar chart representation of the expression pattern of MALAT-1gene in all participants (χ^2 = 33.6, P = < 0.001).

DISCUSSION

Prostate cancer remains the second leading cause of cancer-associated mortality [1].The mean values for total, free and percentage PSA in the prostate cancer and benign prostatic hyperplasia participants were statistically significant ($P<0.05$). This is supported by a similar study that reported elevation of PSA in prostate cancer and non-malignant disorders, such as benign prostatic hyperplasia or prostatitis [19].

Recent cases of prostate cancer and malignant lesions have been linked to high PSA values and a greater percentage revealed histologically to have malignant potential [20]. Long noncoding RNA has been recently demonstrated to play a critical role in prostate carcinogenesis. The aberrant expression of long noncoding RNA in prostate cancer patients is strongly associated with diagnosis, prognosis and clinical treatment [21].

The expression level of MALAT-1 gene showed that 26 prostate cancer participants expressed the gene while among the benign prostatic hyperplasia participants only 7 expressed the gene. The expression level among participants was highly significant. This is supported by a study that confirmed the overexpression of MALAT-1 gene in human hepatocellular carcinoma, breast cancer, pancreatic cancer, colon cancer and prostate cancer [23].

A previous study also reported MALAT-1 to be overexpressed during prostate cancer progression [24]. In early studies, fragments from different regions of the MALAT-1 transcript were detected at higher copy numbers in the plasma of patients with prostate cancer than in non-prostate cancer patients [25]. These data indicate that MALAT-1 is a promising biomarker for prostate cancer detection.

Our results suggests that MALAT-1 is a promising biomarker for predicting prostate cancer risk as revealed by a higher AUC. This is supported by a study that showed a higher AUC for MALAT-1 of 0.670 by ROC analysis compared to value obtained for total PSA (0.545) and percentage free PSA (0.622) [26].

In conclusion, the diagnostic utility of different biomarkers used for prostate cancer diagnosis as revealed by the receiver operating characteristics curve showed MALAT-1 gene as a better biomarker for prostate cancer risk, diagnosis and prognosis.

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BOOK REVIEW

Reviewed by Mike Legge

Lithium: A doctor, a drug, and a breakthrough

Author: Walter A Brown

Publisher: Liveright Publishing Corporation, New York, USA, 2019

The author is a practicing psychiatrist and a Professor at Brown University, USA who help pioneer the use of lithium for the treatment of bipolar disorders and manic depression. Opening with a historical discussion relating to bipolar disorders and manic depression the scene is set for the continued lack of treatment up to relatively modern times. The book considers the discovery of lithium as a therapeutic agent and the issues relating to its use and the major issues in having it accepted into mainstream psychiatry. It is a remarkable story starting with John Cade, an Australian psychiatrist, who was not a researcher, did not have laboratory facilities but was a remarkable observer of events and effects. He was a great believer if there was and idea it had to be tested to see what happened. In the mid-nineteenth century it was noted that lithium would combine with urate in-vitro and made it's entrance into medical practice as a treatment for gout. Progressively lithium became the panacea for a wide range of common medical issues becoming a universal remedy until it was shown to be ineffective and toxic. However, in its heyday there were lithium baths, lithium salts and even lithium beer. Most notably was the "Bib-labeled lithiated lemon, soda and lime" which contained lithium citrate, which became "7-UP" and the lithium was removed by 1950 (Note the atomic weight of lithium is 6.941 which approximates 7!). Because of the popularity of lithium, lithium toxicity became an issue with resulting deaths as the fine line between tolerance and toxicity emerged. This caused the Federal Drug agency (FDA) to place a ban on the use of lithium salts.

In 1945, after returning from the Changi Prisoner of War camp John Cade took a position at Bundorra Repatriation Mental Health Hospital. Here he started his work on manic depression by injecting patient's early morning urine samples into guinea pigs, and notably his guinea pigs died of fits and convulsions. However, the cause eluded him after first considering urea and uric acid toxicity. Increasing the relative proportions of these analytes he found that uric acid would not dissolve and used lithium urate instead. The guinea pigs survived and were lethargic with full recovery in a couple of hours. He then proceeded to try lithium on patients. How the actual dose used is lost but he tried it on himself first for two weeks without toxicity, but remarkably the dose was similar to what the dose is used now. The patients who receive lithium had significant improvement. After publishing his results he received widespread critism from USA and UK psychiatrists as well as the FDA. Lithium toxicity was a major issue from its previous use and abuse. Victor Wynn, an Australian with an interest in measuring biological effects of electrolytes developed the first method for lithium measurement using flame photometry early in 1950, which allowed measurement of therapeutic doses of lithium. However, the use of lithium was still very controversial and a Danish psychiatrist. Morgen Schou decided to conduct his own clinical trials, which proved the success of lithium therapy. However, because lithium occurred as a common salt drug companies could not patent it and they promoted the less effective, the then new anti-psychotic drugs. This became a battle between the pro and anti-lithium psychiatrists and was almost a North –South divide. In 1970 the FDA finally approved lithium, which was already in use in 49 countries by this time for the treatment of bipolar disorders and manic depression.

Commenting on his discovery in a 1970 newspaper article John Cade indicated that how he would have been "absolutely useless as head of a team" preferring to work alone. He died in 1980 and despite the now acknowledged efficacy of lithium the mechanism of its action is still unknown. This book is an excellent read on the development of a major treatment that was almost lost.

Evaluation of two transcutaneous bilirubin devices for the assessment of neonatal jaundice in a diverse New Zealand population

Melanie Adriaansen, Stephanie Williams and Jutta van den Boom

ABSTRACT

Objectives: To assess the accuracy of the Philips Bilichek® and Scanmedics Bilicare™ transcutaneous bilirubin devices against laboratory total serum bilirubin measurements and to ensure the suitability of the devices for use with New Zealand neonates.

Methods: Two devices, BiliChek (Philips), and BiliCare (Scanmedics), were evaluated. Babies requiring a total serum bilirubin also had a transcutaneous bilirubin performed. Exclusion criteria were gestational age <36/40, age <24h or >14d, birth weight <2500g, and infants with complications. Corresponding transcutaneous bilirubin and total serum bilirubin were analysed by correlation and Bland-Altman analysis. Ease of use, and basic cost analysis were also evaluated.

Results: A total of 184 neonates were assessed with 34 neonates excluded (n = 150). Total serum bilirubin results ranged from 84-349µmol/L (mean = 224µmol/L, SD: 53µmol/L). Philips Bilichek® transcutaneous bilirubin with total serum bilirubin demonstrated a correlation co-efficient of r =0.73 (95% CI: 0.65 – 0.80, p<0.001) and had a negative predictive value (NPV) of 98%. Scanmedics Bilicare™ generated similar results (r = 0.7, 95% CI: 0.60 – 0.77, p<0.001, NPV = 96%). Philips Bilichek® user interface had greater ease of use. Both devices had the ability to generate cost savings by avoiding neonatal phlebotomy.

Conclusions: Transcutaneous bilirubin meters can be used for neonatal bilirubin measurements with appropriate treatment thresholds and clinical pathways. Transcutaneous bilirubin meters can replace the visual assessment of jaundice and reduce the number of total serum bilirubin tests required with cost savings to health care providers. Overall, the Bilichek® had a slightly higher correlation and lower variability than the Bilicare™. The Bilichek® was also more user-friendly and therefore was our device of choice.

Keywords: Neonatal jaundice, bilirubin, hyperbilirubinaemia, POCT.

INTRODUCTION

As many as 60% of term and 80% of preterm neonates experience jaundice in their first week postnatally (1,2) and although severe jaundice has serious complications, such as irreversible brain damage (kernicterus) (1,3), the incidence of this is low (4). The UK National Institute for Health and Care Excellence (NICE) jaundice guidelines recommend assessing all neonates for jaundice (1). However, the visual assessment of jaundice, usually performed using the Kramer's rule (5), is not reliable (6). Measuring total serum bilirubin is considered the gold standard (3) but requires a blood sample, usually collected from a heel prick, and sampling is distressing for both neonates and their parents and presents a risk of infection (3). New, non-invasive devices allow for screening of healthy term neonates via transcutaneous bilirubin measurement.

Transcutaneous bilirubin devices are point-of-care-testing devices that analyse the spectrum of light by absorbance and/or reflectance. They use an inbuilt algorithm to quantify the amount of bilirubin present in the neonate's blood stream. Transcutaneous bilirubin devices are increasing in popularity because of the advantages they offer:

- A non-invasive technique that can reduce the need for a heel prick test.
- Ease of use.
- More reliable than visual assessment.
- Cost effective.
- Immediate results (7).
- Easy repeat testing.

The objective of this study was to assess the accuracy of the Philips Bilichek® and Scanmedics Bilicare™ transcutaneous bilirubin devices against laboratory total serum bilirubin measurements in a New Zealand population. The influence of skin tone on the accuracy of transcutaneous bilirubin readings was also assessed. Transcutaneous bilirubin devices have been validated for use on a variety of populations, however, none have been validated with Maori or Polynesian populations. This study was to assess if these devices are suitable for the New Zealand neonatal population. Ease of use and cost analysis was also briefly assessed.

METHODS

Study population

This study was performed at Waitematā District Health Board (Waitematā DHB), Auckland, New Zealand. Waitematā DHB served a population of 615,000 people and had 8,199 deliveries between June 2016 and July 2017. Bilirubin readings were performed in Maternity Suites during the period of research. Neonates ≥36 weeks born by vaginal birth and Caesarean section were accepted for study participation. Neonates of all ethnic backgrounds were included.

The study was granted ethics approval by the Health and Disability Committee, New Zealand. The study was also approved by the Auckland and Waitematā DHB Research Department and the Maori Advisory Committee. Parents provided informed consent for all neonates included in the study. Exclusion criteria were gestational age <36/40 weeks, neonatal age <24h or >14days, birth weight <2500g, and neonates with complications that were admitted to the Special Care Baby Unit.

Skin pigmentation determination

Neonatal skin tone classification was determined by visual observation using the Fitzpatrick Skin Tone Scale (Figure 1). This scale is widely used in dermatology to determine how different skin types react to the sun (8). High quality professional printers were used to print disposable Fitzpatrick Skin Tone Scales, ensuring colour accuracy and consistency.

The scales were held against the neonate's forehead to determine best colour match. Two dedicated medical laboratory scientists performed skin tone classification for all neonates to limit bias. Neonates in categories 1-2 of the Fitzpatrick skin tone scale were classified as 'light' skin tone, neonates in categories 3-4 were classified as 'medium' skin tone, and neonates in categories 5-6 were classified as 'dark' skin tone.

Figure 1. The Fitzpatrick skin tone scale.
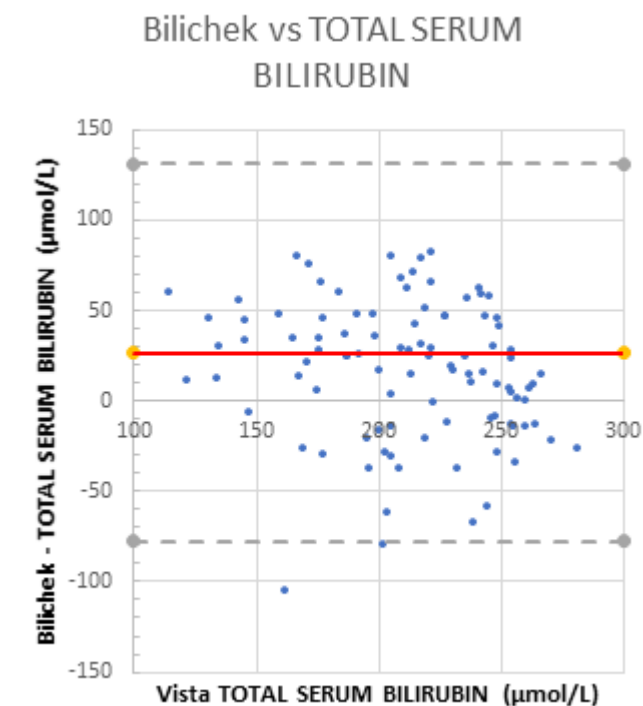
Figure 2. Bland-Altman plot of Bilichek® versus total serum bilirubin.

Device selection and testing procedure
To be included in this study transcutaneous bilirubin devices needed to be portable, handheld, have the ability to scan staff identification and patient barcodes, and have 'connectivity'- the capability of transferring results to electronic medical records giving full traceability. Two devices met these criteria: the Philips Bilichek® and the Scanmedics Bilicare™ (now marketed by Gerium Medical). The specifications of these devices are given in Table 1.

Each neonate was assessed first by the Bilichek® and then by the Bilicare™. Five Bilichek® readings were performed on the neonate's forehead and averaged by the device to give a result. Three Bilicare™ readings were performed on the Scaphoid Fossa of the neonate's ear and averaged by the device to give a result. Readings were performed by two dedicated medical laboratory scientists to reduce variation. Between neonates, device tips were changed for infection control purposes. The Philips Bilichek® performed an automatic calibration with each new tip. The Scanmedics Bilicare™

requested calibration as needed. Heel prick samples were performed by phlebotomy staff following the transcutaneous bilirubin readings. Approximately 400μL of capillary blood was collected from the heel into an ultraviolet protected serum separator tube and tested by the laboratory.

Philips Bilichek®
The Philips Bilichek® transmits white light from a tungsten halogen lamp through the neonate's skin. It measures light that is reflected back to the meter over the visible spectrum of 380nm-760nm at 12nm intervals (1,9). The Bilichek® has an inbuilt algorithm that automatically subtracts light that is reflected by the skins confounding factors- carotenoids, haemoglobin and melanin (9). Using this algorithm, bilirubin is calculated. Because this transcutaneous bilirubin device can correct for melanin, it is considered suitable for an ethnically diverse population (10).

Table 1. Comparison of transcutaneous bilirubin meter specifications.

	Philips Bilichek®	**Scanmedics Bilicare™**
Number of readings per test	Averages 5 readings	Averages 3 readings
Location for reading	Sternum or forehead	Scaphoid Fossa (tip of ear)
User interface	Keypad	Touch screen
Number of results stored	30	40
Gestational age for use	≥ 27 weeks	≥ 35 weeks
Measurement range	0-340μmol/L	0-340μmol/L
Could be used in SCBU/NICU	Yes	Yes
Validated for use with phototherapy	Yes	Not mentioned
Connectivity	Yes	Yes
Scanning	Yes	Yes
Infection control tips	Yes	Yes
Rechargeable batteries	Yes	Yes
High results stored	No	No
Method	Light reflectance	Light transmittance
Manufacturers stated correlation	0.90	0.93

Scanmedics Bilicare™
The Scanmedics Bilicare™ clips to the scaphoid fossa of the neonate's ear and emits light at two visible wavelengths. The device then measures the light transmitted through the ear tissue. As light passes through the ear's tissue, bilirubin molecules absorb the light. The meter takes three readings and calculates the transcutaneous bilirubin using a purpose-designed algorithm (11).

Serum bilirubin

Total serum bilirubin measurements were performed on a Siemens Vista. This method is a colourimetric endpoint test measuring at wavelengths 540 and 700nm. It measures the sum of all four distinct bilirubins that make up the bilirubin total in serum. Mono and diconjugated bilirubin, along with the delta fraction are measured directly. The fourth fraction, unconjugated bilirubin is water insoluble and caffeine is added to the method as an accelerator. This method is a modification of the Doumas method, which itself is a modification of the Jendrassik-Grof method for total bilirubin. The analytical range is 2-428µmol/L. Dilutions were made for samples with higher results. The uncertainty of measurement at 290µmol/L has been calculated as 4% (12).

Statistical analysis

Data was collected and summarised using Microsoft Excel. Calculations were performed using R version 3.5.0, with data treated as normal distributions following Shapiro Wilks testing. Graphs were generated using Microsoft Excel. Due to the small number of neonates with light and dark skin tone results were pooled and analysed. All measurements were treated as continuous data including those >340µmol/L on transcutaneous bilirubin devices (6% of Bilichek® and 3% of Bilicare^{TM} measurements).

RESULTS

A total of 184 measurements were collected with 34 results excluded due to: two neonates <36 weeks, one neonate <24hrs old, six samples without corresponding laboratory results, 15 samples with corresponding laboratory results that had haemolysis flags and 10 samples with a collection time >3hrs between transcutaneous bilirubin reading and laboratory testing. There were no outliers, and the remaining 150 samples were analysed.

The maximum time between the collection of blood and transcutaneous bilirubin reading was approximately three hours (175 minutes), with a mean time difference of 33 minutes. Of the 150 samples, 31 neonates were on phototherapy with the lights turned off for 10 minutes before transcutaneous bilirubin readings were performed. Phototherapy units used at Waitematā DHB include conventional overhead lamps, GE Healthcare Bilisofts^{TM} and Medela Bilibeds^{TM}.

By maternal declared ethnicity most neonates identified as East Asian (36%), and New Zealand European/European (28%) (Table 2). Further demographic data for the study cohort is given in Table 3. The majority of neonates had medium skin tone (63%), with 30% having a light skin tone and 7% a dark skin tone. There were insufficient numbers of neonates in the light and dark skin tone groups to perform statistical analyses on individual groups. Results were pooled to form one group and the overall performance of the Philips Bilichek® and Scanmedics Bilicare^{TM} devices was assessed against laboratory total serum bilirubin testing.

Total serum bilirubin results ranged from 84-349µmol/L with a mean of 224µmol/L (SD: 53µmol/L). Bilichek® transcutaneous bilirubin with total serum bilirubin demonstrated a correlation co-efficient of r =0.73 (95% CI: 0.65 – 0.80, p<0.001). Bilicare^{TM} correlation with total serum bilirubin was r = 0.70 (95% CI: 0.60 – 0.77, p<0.001). However, the co-efficient of non-determination, (or 1- r^2), was 0.54 for the Bilichek® and 0.49 for the Bilicare^{TM}, indicating that approximately half of the variance seen in the transcutaneous bilirubin readings is unrelated to bilirubin levels.

The Bland-Altman plots (Figures 2 and 3) compared the mean and difference of the transcutaneous bilirubin and total serum bilirubin concentrations for each pair of results and demonstrated a positive bias for both the Bilichek® and Bilicare^{TM}. The Bilichek® mean transcutaneous bilirubin -total serum bilirubin difference was 26.67µmol/L, with the values

centred around the mean. The Bilicare^{TM} mean transcutaneous bilirubin total serum bilirubin difference was 6.80µnol/L, with a greater spread than for the Bilichek®.
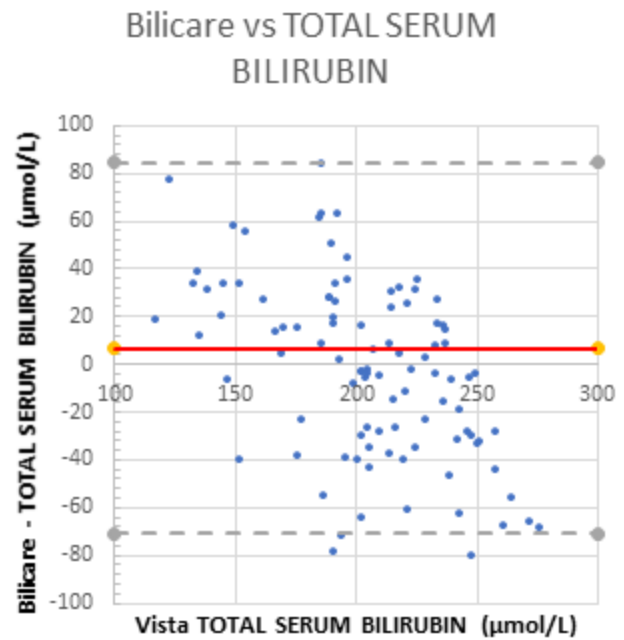
Figure 3. Bland-Altman plot of Bilicare^{TM} versus total serum bilirubin.

At a cut off value of 250µmol/L the Philips Bilichek® had a negative predictive value of 98%, meaning that the device could detect 98% of true negative results. This compared with the Scanmedics Bilicare^{TM} which had a negative predictive value of 96%. The sensitivity, specificity, negative predictive values and positive predictive values are given in Table 4.

The cost of performing a bilirubin measurement on a transcutaneous bilirubin meter is NZ$2-3 for purchasing the infection control tips. This is roughly equivalent to a laboratory total serum bilirubin at NZ$2.02. However, transcutaneous bilirubin measurements do not have the additional cost of performing phlebotomy and cost savings can be provided through this mechanism. At Waitematā DHB a phlebotomy fee is approximately NZ$22.55, leading to possible savings of between NZ$5,000 – NZ$10,000 per annum (Table 5).

Table 2. Maternal declared ethnicity

Region	Number (%)
East Asian (Chinese, Korean, Japanese, Taiwanese)	54 (36.0%)
New Zealand European and other European	42 (28.0%)
South East Asian (Philippino)	18 (12.0%)
South Asian (Indian, Sri Lankan)	17 (11.3%)
Maori and Pacific Peoples (Maori, Samoan, Tongan)	16 (10.6%)
Middle Eastern (Iranian)	2 (1.3%)
Latin America (Argentinian)	1 (0.7%)
Total	150 (99.9%)

Table 3. Demographics of study cohort

	(*n* = 150)
Male	82
Female	68
Birth weight (g) (mean ±SD, (range))	3360 ±0.48 (2.52-4.73)
Age (hours) (mean±SD)	90.5 ±42.5
Skin tone: light (%)	45 (30%)
Skin tone: medium (%)	95 (63%)
Skin tone: dark (%)	11 (7%)
On phototherapy	31 (21%)

Table 4. Sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) at a cut off value of 250µmol/L.

	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)
Philips Bilichek®	82	74	98	20
Scanmedics Bilicare	55	86	96	24

Table 5. Transcutaneous bilirubin meter performance at different cut off values and estimated cost savings.

	Transcutaneous bilirubin device	Cut off value	Neonates missed (%)	Total serum bilirubins that could be prevented (%)	Estimated total serum bilirubins saved per annum	Estimated $ savings per annum per 1,000 neonates*	Estimated neonates missed per annum
UK NICE Guidelines	Bilichek®	250µmol/L	2 (1.3%)	69 (46%)	1551	10,375.27	44
	Bilicare™		5 (3.3%)	102 (68%)	2292	15,332.13	111
Waitematā study results	Bilichek®	200µmol/L	0	28 (19%)	640	4,281.222	0
	Bilicare™		0	39 (26%)	876	5,859.923	0

*Calculations based on 3371 neonatal bilirubins performed by Waitematā Laboratories in 2017 at a phlebotomy fee of NZD22.55.

DISCUSSION

The Pearson correlation coefficient for all neonates was similar between devices. For the Bilichek® with total serum bilirubin r = 0.73. For the Bilicare™ with total serum bilirubin r = 0.70. Both devices had correlations lower than the manufacturer claims (Table 1). The reasons for these discrepancies are not known. Previous studies involving the Bilichek® have found transcutaneous bilirubin correlations with total serum bilirubin values to be between 0.75 – 0.97 (10,13).

Bland-Altman analysis found that both devices had a positive bias. A positive bias reduces the number of false negatives and is ideal for screening tools where laboratory bloods are used as confirmation. The Bilichek® had greater positive bias than the Bilicare™, however, the Bilicare™ had more spread than the Bilichek®. The high negative predictive value for both devices indicates that 96-98% of neonates requiring treatment for jaundice would be captured by the devices if a cut off value of 250µmol/L was used (for transcutaneous bilirubin readings >250µmol/L neonates would require laboratory bloods to be performed). The low positive predictive value of 20-24% indicated that these devices are only useful as screening tools.

The results of this study have confirmed that transcutaneous bilirubin indication of jaundice should be followed by confirmation with total serum bilirubin levels to make treatment decisions (Figure 4).

In our data set, removing neonates on phototherapy from the analysis increased correlation for the Bilichek® but decreased correlation for the Bilicare™ (r = 0.75 and r = 0.69 respectively). Previous studies have reported that phototherapy decreases correlation between transcutaneous bilirubin and blood bilirubin measurements (13). Transcutaneous bilirubin meters should not be used to determine when to start or stop phototherapy and this decision should only be made using total serum bilirubin results (1).

The UK National Institute for Health and Care Excellence (NICE) guidelines (14) recommend performing a total serum bilirubin when the transcutaneous bilirubin measurement is >250µmol/L. Similarly, the American Academy for Paediatrics (APP) (15) suggest a transcutaneous bilirubin can be safely performed when the total serum bilirubin is <15mg/dL (257µmol/L). At a cut off of 250µmol/L in the present study, missed treatment would have occurred for two (1.3%) neonates

with the Bilichek® and for five (3.3%) neonates with the BilicareTM. A cut off value of 200µmol/L would be necessary to capture all neonates requiring treatment but results in an increased number of neonates unnecessarily receiving heel pricks and therefore decreased sensitivity (Table 5).

Despite their advantages, transcutaneous bilirubin meters have not been validated for use with total serum bilirubin nomograms and there is concern about the number of false negatives arising from this practice (16).

Total serum bilirubin nomograms are not designed for transcutaneous bilirubin readings (1). Several studies have proposed transcutaneous bilirubin specific nomograms (1,5) and there is some evidence that plotting transcutaneous bilirubin measurements on transcutaneous bilirubin specific nomograms provides better outcomes (16). Transcutaneous bilirubin specific nomograms are not currently recommended by NICE or AAP and ideally hospitals should be trialling devices to determine their own cut off values before use. Guidelines for the appropriate use of the transcutaneous bilirubin meters should indicate when to perform a total serum bilirubin. An example of appropriate workflow has been provided by Canterbury Health Laboratories in Figure 4.

Signs of jaundice <24 hours of age	→	Perform total serum bilirubin
Signs of jaundice >24 hours of age & Risk factors (maternal antibodies; history of G6PD)	→	**Perform total serum bilirubin**
Signs of jaundice 24-48 hours of age Well infant / no risk factors	→	If Transcutaneous bilirubin measurement > 140µmol/L **Perform total serum bilirubin**
Signs of jaundice 48-72 hours of age Well infant / no risk factors	→	If Transcutaneous bilirubin measurement > 200µmol/L **Perform total serum bilirubin**
Signs of jaundice >72 hours of age Well infant / no risk factors	→	If transcutaneous bilirubin measurement > 250µmol/L **Perform total serum bilirubin**
Phototherapy using the Medela Bilibed ™ Well infant / no other risk factors	→	**Perform SBR** – if level falling Monitor jaundice using transcutaneous bilirubin

Figure 4. An example of guidelines for the appropriate use of transcutaneous bilirubin meters as a screening tool for laboratory total serum bilirubin testing provided by Canterbury Health Laboratories.

Previous studies have demonstrated that screening with transcutaneous bilirubin devices can lower the number of total serum bilirubin measurements performed (1) by up to 34% (17). From our study, introducing transcutaneous bilirubin meters in to Waitematā DHB has the potential to give a 19% reduction in total serum bilirubin measurements when using the Bilichek® device at the more conservative cut off value at 200µmol/L.

This could result in estimated annual savings of NZ$4,000 to NZ$6,000 per 1,000 babies (Table 5). Transcutaneous bilirubin meters are not currently accurate enough to eliminate total serum bilirubin readings, but their primary purpose should be to replace visual assessment, allowing clinicians to make better decisions about which neonates require a heel prick and total serum bilirubin test. Transcutaneous bilirubin meters also allow for easy repeat testing on neonates if there is doubt or follow up is required.

Ease of use of the devices was also assessed by two medical laboratory scientists. For the Bilichek® transcutaneous bilirubin readings were measured on the neonate's forehead whilst the neonate was sleeping. This was easy to perform without disrupting the neonate. We found the device's scanner was accurate and the keypad was sensitive to touch, even with gloves on. Comparatively, the BilicareTM transcutaneous bilirubin readings were performed on the scaphoid fossa of the neonates' ear and some neonates found this discomforting.

Furthermore, the shape of some ears made it challenging to correctly attach the BilicareTM device. The BilicareTM has a touch screen that required firm pressing to activate and the barcode scanner was difficult to use because of its positioning under the device. Overall, we found the Bilichek® device more user-friendly. It should be noted that neither device recorded results >340µmol/L. This is not ideal because not all results are traceable or could be recalled.

Limitations of the study
This study was limited by the number of neonates involved, particularly the number of light skin-tone and dark skin-tone neonates. Inter-batch and inter-device precisions were not measured as part of this study. Accuracy of the meters may be further reduced when introducing multiple users to take transcutaneous bilirubin readings. Ideally, the use of transcutaneous bilirubin meters for neonates on phototherapy should be verified in a separate study.

CONCLUSIONS

Transcutaneous bilirubin meters can be used for neonatal bilirubin measurements with appropriate treatment thresholds and clinical pathways. Transcutaneous bilirubin meters can reduce the number of total serum bilirubin tests required in comparison to performing visual assessment with cost savings to health care providers. It is recommended that hospitals trial devices to determine their own appropriate cut-off values before use. Overall, the Bilichek® had a slightly higher correlation and lower variability than the BilicareTM. The Bilichek® was also more user-friendly and therefore is our device of choice. No conclusions could be drawn about skin colour due to small sample sizes in the light and dark skin tone groups.

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Expression of Interest for Editor of the New Zealand Journal of Medical Laboratory Science

The NZIMLS Council hereby calls for expression of interest from NZIMLS members to succeed Rob Siebers as Editor of the New Zealand Journal of Medical Laboratory Science. Rob has been Editor since 1994 and it is timely to begin succession planning for this position. The successful person would join the Editorial Board as Deputy Editor and over a period of time would learn, under the guidance of Rob, all aspects of editorial work associated with the Journal. The Deputy Editor would take over as Editor when fully conversant with this role. The Editor will be a contractor to the NZIMLS for which remuneration will be offered. The time commitment is approximately six hours per week.

The Journal has been continuously in existence since 1948 and currently is published three times a year (April, August, and November). All submissions are peer reviewed and last year 13 articles plus a number of editorials were published.

To register your expression of interest, please write to the NZIMLS Executive Officer at fran@nzimls.org.nz. Include your current position and work place, why you are interested in this role, your experience in publishing scientific articles. Please also submit a CV. Further information on this position can be obtained from Rob at rob.siebers@otago.ac.nz.

Management review input checklist for ISO 15189:2012 internal auditing: an optimisation guide for medical laboratories

Dennis Mok, Rana Nabulsi and Sharfuddin Chowdhury

ABSTRACT

Objectives: The primary aim of this study was to develop an analytical tool based on conformance requirements (CRs) identified in ISO 15189:2012, which can be used by internal auditors to evaluate the extent of management review input information as specified in Subclause 4.15.2 (Review input) of ISO 15189:2012.

Methods: The CRs were identified in Subclause 4.15.2 of ISO 15189:2012 and its referred subclauses for quantification purposes by content analysis.

Results: A total of 25 CRs were identified in Subclause 4.15.2 of ISO 15189:2012 and these 25/399 (6.3%) CRs are distributed in the 'strategic management' stage of the ISO 15189:2012 process-based quality management system model. A further 252 CRs were identified in Subclause 4.15.2 of ISO 15189:2012 referred subclauses. These 26/477 (5.5%) CRs are distributed in the 'process control, design and planning' stage and 226/252 (90%) CRs are in the 'process evaluation and improvement' stage. The results were used for the development of management review input checklists and a conformity status map to support the interpretation.

Conclusions: The application of a quantitative approach to facilitate internal auditing of the effectiveness of management review performance should improve the quality of information that feeds into the management review which, in turn, influences the crafting of organisational strategy.

Key words: checklist; internal audit; ISO 15189:2012; management review; quality management.

Supplementary material for this article may be found at https://www.nzimls.org.nz/journals-recent.html

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INTRODUCTION

The pathology services industry constitutes a significant capability in the provision of diagnostic information for the diagnosis, monitoring and treatment of health conditions. The services are integrated with other health elements and are intended to conform to the highest levels of professional ethics (1), practices (2) and standards (3). The delivery of services is performed by the medical laboratory, which is structured to provide specific diagnostic information within its areas of responsibility and to contribute as a specialised component of the broader health logistics system. The structure of a modern medical laboratory is relatively complex and requires significant logistic support to operate effectively and efficiently. A marked characteristic of the medical laboratory is the level of commitment to deliver the best standards of health support care in all situations. The contemporary medical laboratory aims to demonstrate its competence and quality by implementation of a relevant international consensus management system standard.

International organisations continue to provide relevant standards that can be implemented by the medical laboratory to demonstrate its ability to operate in conformance to specifications, such as the European Committee for Standardization (4,p.902) and the International Organization for Standardization (ISO) (4,pp.1874-1875). The implementation of management system standards developed by the ISO enables the medical laboratory to enhance its productivity and maximise the benefits derived from the application of those standards (5). One such specific management system standard is ISO 15189:2012 (6) that remains a hallmark of the medical laboratory's ability to provide high quality of service (7). ISO 15189:2012 specifies the minimum necessary activities and requirements to deliver competent medical laboratory services (8,9). The competent implementation of ISO 15189:2012 and accreditation by an accreditation body represent a significant achievement in raising confidence, expectation and morale within a combined health system.

The implementation of ISO 15189:2012 requires innovative strategic quality management considerations in response to the changes in the marketplace. These changes can commonly range from hard (technical) to soft (human) aspects, such as regulatory updates (10) and physical structures (11), to more challenging aspects of cultural change (12). Despite the ever-changing environment, well-structured strategic management can support the medical laboratory in crafting strategy that aligns with the medical laboratory's capabilities and resources (13). Overall, strategic management fulfils a critical role in the ISO 15189:2012 process-based quality management system model (14) (Figure 1) and is a support activity in the value chain of the medical laboratory (15,16). The medical laboratory needs to craft and make strategic choices in order to meet all strategically relevant prerequisites in preparation for the management review process.

Given the relative importance of strategic management in the medical laboratory, it should be a priority for resources to concentrate on the management review process. The strategically relevant factors can also be used in support of risk management (17). Although the process of strategic management via management review is crucial to the medical laboratory quality management system, the term 'management review' remains undefined by the ISO; however, the term 'management' is clearly equivalent to 'laboratory management' and synonymous with the term 'top management' (6), and laboratory management has been defined by the ISO as 'person(s) who direct and manage the activities of a laboratory' in Subclause 3.10 of ISO 15189:2012 (6,p.3). In addition, the term 'review' has been defined by the ISO as 'determination of the suitability, adequacy or effectiveness of an object to achieve established objectives' in Subclause 3.11.2 of ISO 9001:2015 (18,p.27). Nevertheless, it is apparent that prior to conducting a management review, a range of results must be collected from evaluations of various aspects of the medical laboratory's operations, as specified in Subclause 4.15 (Management review) of ISO 15189:2012 (6,pp.18-19). Overall, there is a

strong linkage between the sufficiency of management review input and the effectiveness of management review performance.

Figure 1. The ISO 15189:2012 process‑based quality management system model. The four circles represent the major stages of ISO 15189:2012 processes: the strategic management stage; the process control, design and planning stage; the analytical processes stage; and the process evaluation and improvement stage.
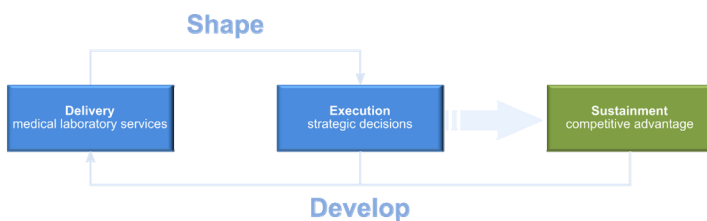
Figure 2. The relationship between resource endowments, strategies and sustained competitive advantage. Competent medical laboratory services are delivered by organisations that are confirmed by an accreditation body that operates according to ISO/IEC 17011:2017. The service delivery supports the shaping the strategic decisions, especially in the areas of adequacy, effectiveness, suitability and supports the care of patients as specified in Subclause 4.15.1 (General) of ISO 15189:2012. Specific competitive advantage is crafted with the competent execution of strategic decisions.

Thus, so far there has been no quantitative analytical tool, such as a conformance management checklist, available for internal auditors to evaluate the relevant management review input factors that constitute the main source of input for the management review process in ISO 15189:2012. Although an attempt using a qualitative approach has been made to analyse the review input points (19), unfortunately the study could not produce a conclusive outcome at the completion of analysis. The present study is based on a quantitative approach and the checklists produced should enable the internal auditors to evaluate the extent of usage of management review input information as listed in Subclause 4.15.2 (Review input) of ISO 15189:2012 (6,pp.18‑19) in an accurate manner. Although it has been determined that Subclause 4.15.2 (Review input) of ISO 15189:2012 has 25 CRs (20) and refers to more subclauses in Clauses 4 (Management requirements) and 5

(Technical requirements) of ISO 15189:2012 (6,pp.6‑39), the internal audit process should evaluate the cross‑referenced CRs to ensure comprehensive coverage of such management review input factors. This action should enable a more detailed situational awareness of the effectiveness of the management review performance through the linkage to sufficiency of management review input information, and this in turn should enable improvement of the relevant processes that shape the organisational strategy.

The study described in this paper sought to achieve a comprehensive evaluation of management review input and comprised two main steps. First, a distribution analysis of CRs was performed for Subclause 4.15.2 of ISO 15189:2012 for quantification purposes by content analysis. The results were required for the development of management review input checklists. Second, a distribution analysis of CRs was performed for Subclause 4.15.2 of ISO 15189:2012 referred subclauses. This was performed for Subclauses 4.15.2 a) to 4.14.2 o) of ISO 15189:2012 (6,pp.18‑19). The results were required for the development of a CRs checklist for Subclause 4.15.2 of ISO 15189:2012 referred subclauses. Finally, a management review input conformity status map was developed to aid in the interpretation of results of quantitative analysis of Subclause 4.15.2 of ISO 15189:2012. The findings should make a useful contribution to the field of internal auditing, especially to strategic management evaluation. The only practical constraint for the proposed evaluation methodology is whether the medical laboratory has competent internal auditors to identify the required information as set out by Clause 7 (Competence and evaluation of auditors) of ISO 19011:2018 (21,pp.28‑34).

MATERIALS AND METHODS
Content analysis of Subclause 4.15.2 (Review input) of ISO 15189:2012
The content analysis was performed using ISO 15189:2012 published by the ISO. The specific areas of interest for analysis were primarily in Subclause 4.15.2 of ISO 15189:2012 and the referred subclauses in ISO 15189:2012.

Quantitative analysis of conformance requirements of Subclause 4.15.2 (Review input) of ISO 15189:2012
To establish specific audit criteria that could be performed against, a computer‑aided qualitative data analysis package, NVivo 10 for Windows (version 10.0.638.0) (QSR International, Doncaster, Victoria), was used for the quantitation of CRs during the content analysis (22). The CRs were elicited using NVivo 10 and a previously described procedure (20). The same approach to quantitation as applied to ISO 15189:2012 and ISO 22870:2016 has been detailed elsewhere (20,23).

RESULTS
Quantitation of Subclause 4.15.2 of ISO 15189:2012 conformance requirements
Content analysis was used to identify the CRs in Subclause 4.15.2 of ISO 15189:2012 (Table S1). Subclause 4.15.2 of ISO 15189:2012 contains a total of 25 CRs. The overall range was 1/25 (4%) CR to 4/25 (16%) CRs in Subclause 4.15.2 n) of ISO 15189:2012 (Table S1).

Subclause 4.15.2 of ISO 15189:2012 refers to further specific subclauses (*n*=14) in Subclauses 4.15.2 a) to 4.15.2 l) of ISO 15189:2012 (6,p.18) and listed according to the format of Subclause 25.4 (Referencing) of ISO/IEC DIR 2:2018 (24,p.52). These referred subclauses were analysed and found to contain a total of 252 CRs (Tables S2 and S3).

The frequency of Subclause 4.15.2 of ISO 15189:2012 conformance requirements in the ISO 15189:2012 process‑based quality management system model

Subclause 4.15.2 of ISO 15189:2012 contains 25/1 515 (1.7%) CRs relative to Clauses 4 and 5 of ISO 15189:2012 and these 25 CRs are distributed in the 'strategic management' stage of the ISO 15189:2012 process‑based quality management system model (Figure 1) with results of 25/399 (6.3%) CRs (Figure S1).

Subclause 4.15.2 of ISO 15189:2012 referred subclauses (*n*=14) contain 252/1 515 (17%) CRs relative to Clauses 4 and 5 of ISO 15189:2012 and these 252 CRs are distributed in the 'process control, design and planning' stage with 26/477 (5.5%) CRs (Figure S2) and the 'process evaluation and improvement' stage with 226/252 (90%) CRs (Figure S3).

The frequency of Subclause 4.15.2 of ISO 15189:2012 conformance requirements in the value chain model

Subclause 4.15.2 of ISO 15189:2012 operates within the strategic management stage of the ISO 15189:2012 process‑based quality management system model which fits within the 'support activities and costs' of the value chain model (15) (Figure S4). The CRs of Subclause 4.15.2 of ISO 15189:2012 and its referred subclauses are distributed within the support activities and costs of the value chain (Figure S4).

Conformance requirement checklist for Subclause 4.15.2 of ISO 15189:2012

The CR checklist was developed using the CRs (*n*=252) of Subclause 4.15.2 of ISO 15189:2012 (Figure S5). The frequencies of CRs expressed as percentages ranged from 0/252 (0%) CR in Subclauses 4.15.2 m) to 4.15.2 o) of ISO 15189:2012 (6,pp.18‑19) to 54/252 (21%) CRs in Subclause 4.15.2 l) of ISO 15189:2012 (6,p.18) (Table S2).

Management review input checklist for Subclause 4.15 of ISO 15189:2012

The management review input checklist was developed using the CRs (*n*=25) of Subclause 4.15 of ISO 15189:2012 (Figure S6). The frequencies of CRs expressed as percentages ranged from 1/25 (4%) CR to 4/25 (16%) CRs (Table S1).

Management review input conformity status map for Subclause 4.15 of ISO 15189:2012

The management review input conformity status map was developed for interpretation of results using colour‑coded grading in three colours (Figure S7). Green highlights that the management review input checklist shows a total coverage of 100% and 'the medical laboratory is highly likely to make excellent progress and to achieve strategic management objectives'. Amber highlights that the management review input checklist shows a total coverage of 50% to 99% and 'the medical laboratory has the potential to make good progress and to achieve planned strategic management deliverables by addressing shortfalls'. Red highlights that the management review input checklist shows a total coverage of ≤ 49% and 'the medical laboratory is highly likely to operate in a strategic risk setting unacceptable to the current medical laboratory quality management system'.

DISCUSSION

This paper is primarily concerned with the optimisation of management review process in order to provide laboratory management with supportable and viable information for informed strategic decision‑making. This was achieved by the development of practical checklists for medical laboratory professionals to perform internal audits with the intent of covering all possible considerations as specified in

Subclause 4.15.2 of ISO 15189:2012. The results showed that Subclause 4.15.2 of ISO 15189:2012 contained its own CRs as well as additional CRs from its referred subclauses. The elicitation of these CRs enable the internal auditor to ensure comprehensive management review input results are included for evaluations by laboratory management. The proposed work documents, CR checklist for Subclause 4.15.2 of ISO 15189:2012 (Figure S5) and management review input checklist (Figure S6), should be used by internal auditors who have had training in auditing against ISO 15189:2012 in accordance with ISO 19011:2018 (21) in order to obtain optimal productivity. Overall, the analysis of CRs in Subclause 4.15.2 of ISO 15189:2012 has the potential to enhance continual improvement and optimisation of the management review process.

The medical laboratory conducts routine internal audits to determine whether all activities in the quality management system are meeting specifications of the medical laboratory as well as Clauses 4 and 5 of ISO 15189:2012. These auditing actions are imperative for the medical laboratory quality management system that intends to ensure that all practices conform to the specifications and, if required, implement corrective action. The ISO's recommended way to conduct such internal audit activities is detailed in Clause 6 (Conducting an audit) of ISO 19011:2018 (21,pp.18‑28) followed by a document review of the relevant areas of interest as specified in Subclause 6.3.1 (Performing review of documented information) of ISO 19011:2018 (21,p.19). The document review is also a structured activity and should be performed using evaluation checklists (25). More specifically, the proposed checklists (Figures S5 and S6) enable medical laboratory professionals to conduct internal audits with a similarly consistent approach. This particular format of work document is highly likely to add value to the medical laboratory when completed by medical laboratory professionals.

An implication of this is the possibility that effective internal audits can add value to the medical laboratory quality management system by making contributions directly to the strategic management level. The contributions can shape the way medical laboratory makes competent strategic management decisions. However, this can be achieved more effectively and efficiently if trained medical laboratory professionals are used for performing auditing activities. This is more probable when the medical laboratory professionals have received effective auditing training and are deemed competent in internal auditing (26). This particular requirement is actually specified in Subclause 5.1.5 (Training) of ISO 15189:2012 (6,p.20) which states that training needs to be provided by the medical laboratory if they are assigned for evaluation and internal audit processes. Three potential implications when assigning medical laboratory professionals for performing internal auditing should be noted. First, when medical laboratory professionals from different disciplines are used to perform audits in order to enhance impartiality and objectivity of the internal audit process, the proposed checklists (Figures S5 and S6) are highly likely to enable them to provide consistent analytical judgement and productivity; despite the possibility of the audit being conducted by personnel who normally work in another scientific discipline. The checklists were developed according to the finding of 1 515 CRs in Clauses 4 and 5 of ISO 15189:2012 in a previous study (20) which can counter‑balance the susceptibility to restricted technical expertise (27). The same CRs were used to support the development of checklists enabling the audit to adopt a consistent and measurable approach. Second, when medical laboratory professionals use the guidance principles of ISO 19011:2018 to perform audits using the proposed checklists, the combination of the auditing skills with the checklists is highly likely to ensure all audit activities are leading to a high level of reliability and validity of task performance.

It is very important for the internal auditors to gather as much relevant information in the shortest possible timeframe. This can only be achieved by using the right audit methods according to the audit plan. The routine practice of these skills by trained internal auditors is likely to enable them to include improvement and maintenance of auditing competence as part of their personal development plan (28), as recommended in Subclause 7.6 (Maintaining and improving auditor competence) of ISO 19011:2018 (21,p.34) as well as meeting the CRs of Subclause 5.1.8 (Continuing education and professional development) of ISO 15189:2012 (6,p.21). Third, when medical laboratory professionals who work routinely in technical fields are used to perform audits in management system requirements of the medical laboratory, which is the main content of Subclause 4.15.2 of ISO 15189:2012, then they are highly likely to obtain further situational awareness of the operational aspects of the medical laboratory. Medical laboratory professionals who practise at the bench are not normally exposed to the management aspects of the medical laboratory. The enhancement of situational awareness is particularly important to the corporate knowledge management of the organisation (28). Overall, these advantages are likely to empower internal auditors to achieve optimal outcomes in addressing the areas of vulnerability and identifying opportunities for improvement in the medical laboratory quality management system.

CONCLUSIONS

When suitable ways to conduct management reviews are available to the medical laboratory, it should be possible to determine what is reasonably practicable to make optimum use of available resources to maximise management review productivity. The level of conformity for the input can be visualised using the management review input conformity status map, which displays the results using a three‑colour colour‑coded grading (Figure S7). This is to aid internal auditors in the creation of practical visualisations that can be used for reporting purposes to laboratory management. It is the ultimate aim of the medical laboratory to obtain a green grade because it represents a coverage rate of 100% with high probability of making excellent progress in achieving the planned strategic management objectives. The achievement offers a firm foundation for the medical laboratory to obtain meaningful information for the review analysis as specified in Subclause 4.15.3 (Review activities) of ISO 15189:2012 (6,p.19). The foundation contributes directly to the support activities and costs of the medical laboratory value chain (Figure S4). The value chain offers an explanation for the importance of conformity allowing strategic decision‑making in the contemporary setting of rapid change (29). The ultimate aim is to execute strategic decisions that can sustain competitive advantage in contributing to patient care (Figure 2).

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2020 ANNUAL SCIENTIFIC MEETING SPEAKER PROFILES

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Distinguished Professor Harding is a University Distinguished Professor, and a researcher in the LiFEPATH research group of the University's Liggins Institute. Her training included a medical degree at the University of Auckland, a D Phil at the University of Oxford, and a postdoctoral Fogarty Fellowship at the University of California at San Francisco.

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Her on-going research concerns the role of nutrition and growth factors in the regulation of growth before and after birth, blood glucose regulation in the newborn, and the longterm consequences of treatments given around the time of birth.

Amongst her many awards are the Howard Williams Medal from the Royal Australasian College of Physicians, the Beaven Medal from the Health Research Council of New Zealand, the Norman J Siegel Outstanding Science Award from the American Pediatric Society, and the Rutherford Medal from the Royal Society of New Zealand.

Dr Ken Dutton-Regester

Dr Ken Dutton-Regester is a cancer researcher at the QIMR Berghofer Medical Research Institute exploring new ways to treat late-stage melanoma. This includes a partnership with the Broad Institute of Harvard MIT to use CRISPR-knockout screens to identify new drug targets in rare forms of melanoma and understanding the mechanism of drug-resistant transcriptional cell-states.

Ken is currently a Melanoma Research Alliance Young Investigator and AMP Foundation Tomorrow Maker.

In 2019, Ken founded Excite Science to design portable experiential exhibits to explain complex science in fun and creative ways. The first project of Excite Science was to create the world's first cancer biology-themed puzzle/escape room as a unique way to educate people about cancer. The project has attracted significant interest, been hosted by numerous organizations throughout Australia and has been profiled at TEDxUQ and Channel 10's National Science Television program 'Scope'. In 2019, Ken was appointed as a member of the Questacon Advisory Council by invitation by the Minister for Industry, Science and Technology.

Evaluation of a new combination: ceftriaxone-disodium edetate-sulbactam as a broad-spectrum option for multidrug-resistant bacterial infections

Mohd. Shahid, Shariq Ahmed, Zobair Iqbal, Hiba Sami and Anuradha Singh

ABSTRACT

Background: The presence of numerous antibiotic-resistance mechanisms in Gram-positive and Gram-negative bacteria is a global concern, which is further complicated by emergence of newer mechanisms in recent years. Few new compounds are in the production-pipeline that show potential for usage as antimicrobial agents. Antibiotic adjuvant ceftriaxone-disodium edetate-sulbactam, available under tradename Elores™, showed potential in this study by demonstrating antimicrobial activity against various multidrug-resistant bacteria.

Methods: In this prospective in-vitro study, we tested the antimicrobial activity of Elores™ against a battery of clinical Gram-positive and Gram-negative bacterial isolates, including antibiotic-susceptible and antibiotic-resistant bacteria such as ESBL-producing Enterobacterales (ESBLPE), carbapenem-resistant Enterobacterales (CRE), Enterobacterales showing colistin-resistance (ECR), methicillin-resistant *Staphylococcus aureus* (MRSA), and vancomycin-resistant Enterococci (VRE).

Results: Elores™ showed excellent activity against the tested Gram-positive and Gram-negative bacterial species, including some highly resistant species such as ESBL-producers, CRE, species resistant to colistin, MRSA and VRE. Elores™ was non-inferior to tigecycline in VRE isolates and non-inferior to colistin in *Escherichia coli*, *Citrobacter* species, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. However, in *Klebsiella* species the activity of Elores™ was notably better than colistin.

Conclusions: In addition to activity against ESBL-producers and CRE, the activity of Elores™ against colistin-resistant Enterobacterales, MRSA and VRE showed promise, indicating its use as a potential candidate for empirical therapy due to its high activity against multidrug-resistant Gram-positive and Gram-negative bacteria.

Keywords: Elores™, antibiotic-resistance mechanisms, Enterobacterales.

INTRODUCTION

Antimicrobial resistance has dramatically increased in recent years to an alarming extent, subsequent to which global initiatives were called upon from different forums to curb this threatening issue, including the endorsement of a global action plan by the World Health Organization (WHO) to curb antimicrobial resistance (1). Recently, in a series (Antimicrobials: access and sustainable effectiveness) published by the *Lancet* had emphasised the need for policy interventions to combat emerging global burden of resistance (2). Confronted with this burning issue, other leading journals also emphasised a pressuring need to develop newer antibiotics (3). The rates of antimicrobial resistance vary geographically, which in many cases is a reflection of selection pressure due to antibiotic prescribing habits. However, in all of these resistance cases the mechanisms may be multiple, including reduced permeability to antibiotics, increased efflux pumps, changes in antibiotic targets by mutation, or modification of target enzymes; all of which are prevalent globally.

However, the spread of the resistance genes through various mobile genetic elements is the common mechanism of acquired resistance (4,5). Few new antibiotics are currently in the pipeline which would otherwise raise some hope in curbing life threatening infections caused by extensively resistant organisms. In such a situation, if a novel compound shows promising results against resistant bacteria, it brings hope for future treatment options. Elores™ (ceftriaxone- disodium edetate- sulbactam) is a promising new agent which is currently patented in many countries, including in the U.S. (the details of the patent numbers in respective countries are given in the

respective methodology section). Recent published data projects this new antibiotics adjuvant as a carbapenem-sparing drug, mainly against Gram-negative bacteria, and demonstrated benefits of using this novel compound empirically in severe illnesses, including ventilator associated pneumonia (6).

However, the potential of Elores™ has not been thoroughly explored against Gram-positive extensively drug-resistant bacteria such as MRSA and VRE, nor against Gram-negative bacteria resistant to colistin, which is generally used as a last resort antibiotic. In this study we evaluated the antimicrobial potential of this antibiotic adjuvant against multidrug-resistant clinical Gram-positive and Gram-negative bacterial isolates, including ESBL-producing- and carbapenem-resistant-Enterobacterales, colistin-resistant Enterobacterales, methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant Enterococci.

METHODS

Study type

This was a prospective in-vitro study performed on routine clinical samples received for culture and sensitivity at the Microbiology Laboratory of the Jawaharlal Nehru Medical College & Hospital Aligarh, India. Ethical clearance was obtained from the Institutional Ethical Committee of the Jawaharlal Nehru Medical College & Hospital.

Elores™ patent details

The patent information provided by the source company Venus Remedies, India is as follows: 236996 (India), 2007/4394 (South Africa), 91204 (Ukraine), 2397768 (Russia), 279582

(Mexico), 2005310888 (Australia), 555075 (New Zealand), 8273732; 13/626, 236 (USA), 10-1244362 (South Korea), 5269415 (Japan), EP1841432 (Europe).

Patients and clinical samples

A total of 111 patients were included in this study from which the respective number of bacterial isolates, including various Gram-negative bacterial species, MRSA and VRE, were obtained after routine culture and sensitivity. These bacterial isolates were further tested for antibacterial activity against Elores™ discs. Gram-negative bacterial isolates were obtained from 67 clinical samples: pus-44; tracheal aspirate-6; sputum-5, blood-4; broncho-alveolar lavage and semen-2 each; cerebrospinal fluid, cervical swab, urine and vaginal swab-1 each. Twenty-seven VRE and 17 MRSA were obtained from urine, pus, blood and abdominal drain specimens. All 27 VRE and 17 MRSA were checked by molecular studies (PCR) for *van*A, *van*B and *mec*A genes respectively as per published procedures (7,8). Sixty three out of 67 Gram-negative isolates were ESBL producers, as determined by the Clinical Laboratory Standards Institute combination disc method (12). Representative isolates of resistant Gram-negative bacterial species were tested for respective molecular mechanism of resistance (bla_{CTX-M}, bla_{ampC} and bla_{NDM-1}) as per the procedures published elsewhere (5,9-11).

Bacterial isolates

The following Gram-negative isolates were obtained from 67 clinical samples: *E. coli* (n=38), *Citrobacter* spp (n=10), *Klebsiella pneumoniae* (n=6), *Pseudomonas aeruginosa* (n=6), *Acinetobacter baumannii* (n=4) and *Klebsiella oxytoca* (n=3). From the remaining 44 samples MRSA and VRE were obtained (MRSA= 17 and VRE= 27 isolates). Ten representative samples that were phenotypically ESBL were genotypically confirmed by the presence of CTX-M gene. Six representative samples, three *Pseudomonas* species and three *Enterobacterales* were tested for NDM-1, but none showed the presence of NDM-1 gene.

Routine culture identification/ sensitivity and Elores™ discs

The bacterial isolates were tested for identification and sensitivity by Kirby-Bauer method and the antibiotic susceptibility results were interpreted as per standard procedures (12,13). For quality control, *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922) and *P. aeruginosa* (NCTC 6749) were used. Representative isolates of Gram-negative bacterial species and all the MRSA and VRE strains were confirmed by automated Vitek-2 compact system (Biomerieux-Diagnostics, USA). For antibiotic susceptibility testing of Gram-negative bacterial species (except *Pseudomonas* spp) the following antibiotics and concentrations were used: amikacin (30 µg), amoxicillin-clavulanate (20/10 µg), cefixime (5 µg), ceftriaxone (30 µg), ceftriaxone-sulbactam (30/15 µg), cotrimoxazole (1.25/23.75 µg), piperacillin-tazobactam (100/10 µg), levofloxacin (5 µg), meropenem (10 µg), colistin (10 µg) and Elores™ (30/15 µg). The antibiotics used for *Pseudomonas* species were: piperacillin-tazobactam (100/10 µg), amikacin (30 µg), aztreonam (30 µg), cefepime (30 µg), ceftazidime (30 µg), meropenem (10 µg), gentamicin (10 µg), levofloxacin (5 µg), colistin (10 µg) and Elores™ (30/15 µg). The antibiotic panel used for MRSA strains was: azithromycin (15 µg), amoxicillin-clavulanate (20/10 µg) (interpreted according to EUCAST) [14], levofloxacin (5 µg), cotrimoxazole (1.25/23.75 µg), clindamycin (2 µg), amikacin (30 µg), cefoxitin (30 µg), vancomycin (30 µg) and Elores™ (30/15 µg). And, for VRE, the following antibiotics were tested: benzyl penicillin (10 units), high content gentamicin (120 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), erythromycin (15 µg), linezolid (30 µg), teicoplanin (30 µg), vancomycin (30 µg), tetracycline (30 µg), nitrofurantoin (300 µg), tigecycline (15 µg) and Elores™ (30/15 µg). Resistance to colistin in Gram-negative bacteria and vancomycin in MRSA and VRE was further confirmed by the automated Vitek-2 system.

In-vitro susceptibilty testing of Elores™ discs

Antibiotics susceptibility of Elores™ discs against the Gram-negative and Gram-positive (MRSA and VRE) isolates was performed by the Kirby-Bauer method (13). Antibiotics discs (45; 30/15 µg) of antibiotic-adjuvant ceftriaxone-disodium edetate-sulbactam were procured from Abtek Biologicals Ltd, Liverpool, United Kingdom. Results were interpreted as per the manufacturer's instructions.

RESULTS

The characteristics of the resistance mechanisms of the Gram-negative isolates tested are given in Tables 1 and 2.

Table 1. Distribution of ESBL-producing Gram-negative isolates.

Organisms		Phenotypic detection for ESBLs	
		Test performed on no. of isolates	ESBL detected in no. of isolates
Enterobacterales		57	55
	Escherichia coli	38	37
	Citrobacter species	10	10
	Klebsiella species	9	8
Pseudomonas species		6	5
Acinetobacter species		4	3

Table 2. Genotypic characterisation of representative isolates.

Isolate no.	Organism	Gene detected
40	*Klebsiella* species	bla_{CTX-M}, bla_{TEM}, bla_{SHV}
11	*Escherichia coli*	bla_{CTX-M}, bla_{ampC}
3	*Klebsiella* species	bla_{CTX-M}, bla_{TEM}, bla_{SHV}
61	*Escherichia coli*	bla_{CTX-M}, bla_{ampC}
36	*Escherichia coli*	bla_{CTX-M}, bla_{ampC}
57	*Escherichia coli*	bla_{CTX-M}, bla_{ampC}
56	*Klebsiella* species	bla_{CTX-M}, bla_{ampC}
59	*Escherichia coli*	bla_{CTX-M}, bla_{ampC}
55	*Escherichia coli*	--------
47	*Citrobacter* species	bla_{CTX-M}
54	*Pseudomonas* species	bla_{SHV}
58	*Pseudomonas* species	----------
38	*Pseudomonas* species	-----------

Antibiotics susceptibility profile and Elores™ activity

Gram-negative bacterial isolates tested in this study were multidrug-resistant including some isolates, such as *Acinetobacter baumannii*, which were resistant to all of the tested antibiotics except colistin (Table 1). Elores™ was non-inferior to colistin in Gram-negative bacterial species (*E. coli*, *Citrobacter* species, *A. baumannii* and *P. aeruginosa*). However, in *Klebsiella* species, the activity of Elores™ was better than colistin (Table 3). All of the vancomycin-resistant *Enterococci* were also resistant to fluoroquinolones tested.

Alarmingly 22.2% (6/27) of VRE were resistant to linezolid. All of the VRE isolates were susceptible to tigecycline, and Elores™ was found non-inferior to tigecycline against VRE. Interestingly, Elores™ was active against the bacterial isolates which harbored complex molecular resistance mechanisms. The detailed antibiotic susceptibility pattern of various antibiotics, including Elores™, against Gram-negative and Gram-positive isolates (MRSA and VRE) is shown in Table 1. The representative in-vitro susceptibility results of elores discs against various bacterial species and the representative resistance mechanisms: ESBL (bla_{CTX-M}/ bla_{ampC}), CRE (bla_{NDM-1}), VRE (bla_{vanA}) and MRSA (bla_{mecA}) are shown in Figure 1. Gram-positive isolates (MRSA and VRE) are shown in Table 3.

Table 3. Antibiotic susceptibility profile (including Elores™) against bacterial species tested.

Organism (n) and antibiotics tested	Resistant percentage (n)	Sensitive percentage (n)
***Enterobacterales* (57)**		
Amikacin	47.37% (27)	52.63 % (30)
Meropenem	49.12% (28)	50.88% (29)
Colistin	7.02% (4)	92.98% (53)
Elores	0% (0)	100% (57)
Acinetobacter (4)		
Amikacin	100 % (0)	0 % (0)
Meropenem	100 % (0)	0 % (0)
Colistin	25 % (1)	75% (3)
Elores	0 % (0)	100% (0)
***Pseudomonas* (6)**		
Amikacin	50 % (3)	50% (3)
Meropenem	83.33 %(5)	16.67% (1)
Colistin	0% (0)	100% (0)
Elores	0% (0)	100 % (0)
VRE (27)		
Ceftriaxone	100 % (27)	0% (0)
Ceftriaxone+sulbactum	100 % (27	0% (0)
Elores	3.70% (1)	96.30% (26)
Linezolid	22.22% (6)	77.78% (21)
Levofloxacin	100 % (0)	0 % (0)
Erythromycin	96.30% (26)	3.70% (1)
Tetracycline	51.85% (14)	48.15 % (13)
Tigecycline	0 % (0)	100 % (27)
MRSA (17)		
Azithromycin	82.35% (14)	17.65% (3)
Levofloxacin	70.59% (12)	29.41 % (5)
Cotrimoxazole	58.82% (10)	41.18% (7)
Clindamycin	35.29% (6)	64.71% (11)
Amikacin	17.65% (3)	82.35 % (14)
Vancomycin	0% (0)	100 % (17)
Elores	0% (0)	100 % (17)
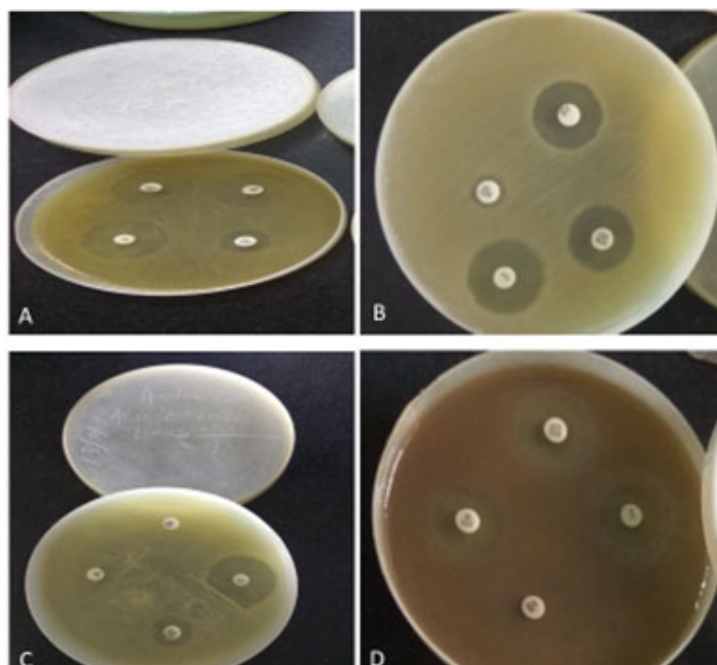
Figure 1. Antibacterial activity of Elores against representative *E. coli* (A), *Klebsiella pneumoniae* (B), *Acinetobacter baumannii* (C) and *Pseudomonas aeruginosa* (D).

DISCUSSION

Antibiotic resistance in Gram-negative bacteria has increased dramatically over the past few decades and the most common mechanism is by the production of beta-lactamases such as extended-spectrum beta-lactamases (15,16). Due to this increase in ESBL-producing isolates in clinical settings, carbapenems were considered empirically or as a tailored-down therapy. However, in recent years a significant volume of clinical isolates have demonstrated the presence of metallo-beta-lactamases, thus causing treatment failure to prescribed carbapenems (17). Due to this increase of emerging extended-resistant isolates there has been a pressured need to prescribe reserved antibiotics such as tigecycline and colistin. However, reports of resistance to these reserved drugs have also emerged in recent years (9,18-23). Emergence of resistance even to these reserved drugs has landed us in a very critical situation seeing that there are not many investigative drugs available in the antibiotic armamentarium (17). Said that, researchers recently have tried exploring potential of using potentiators of the already existing antibiotics in the form of antibiotic-adjuvants (17). Antibiotic-adjuvants refer to molecules which usually do not have antibiotic activity. These adjuvants are combined with antibiotics in order to potentiate the overall activity of the antibiotic-adjuvant entity through various mechanisms as discussed below. One recently developed example is ceftriaxone- disodium edetate- sulbactam (Elores™) which is prepared by combining:

- Antibiotic = ceftriaxone
- ESBL-inhibitor = sulbactam
- Adjuvant = disodium edetate

The antibiotic adjuvant Elores™ was initially patented by the Companies and Intellectual Property Registration Office (CIPRO), South Korea and was marketed back in 2013 by a Korean Pharma company, Goodwills Co (24). Clinical trials have suggested the clinical and microbiological efficacy of Elores™ in ESBL-producing Gram-negative pathogens and few Gram-positive pathogens showing clinical cure rate of as high as 80.3% as opposed to patients treated with ceftriaxone, which showed a cure rate of 30.8% (24). The microbiological efficacy in terms of bacterial eradication was reported as high as 85.3% in contrast to ceftriaxone alone (23.1%) (25).

Various possible mechanisms have been proposed for enhanced activity of Elores[TM] (6), such as:

- Ceftriaxone, sulbactam and EDTA acting synergistically.
- Enhanced activity of the antibiotic-adjuvant due to chelation of divalent ions by EDTA.
- Increased penetration of the antibiotic-adjuvant due to alteration of outer membrane permeability of bacteria.

However, we speculate that though the exact mechanism is not yet clear, it could be a complex mechanism such as the ones stated above plus the individual activities of sulbactam and EDTA, such as:

- Sulbactam showing intrinsic antibacterial activity against certain bacterial species, for instance Acinetobacter (26).
- Antimicrobial activity of native EDTA against pathogenic bacteria (27,28).

It is noteworthy that an anti-biofilm activity of EDTA has recently been reported (27,28). These studies would suggest that incorporating potentiators, such as EDTA, to the existing antibiotic armamentarium could be utilised as an alternative approach to combat antibiotic resistance; at least until we receive newer compounds showing efficient antibacterial activity against extensively- or pan-resistant organisms. In this era of emerging extensive- or pan-resistant organisms, combined with the paucity of available alternatives, the idea of using carbapenem-sparing agents is well understood. Thus, Elores[TM] could widely be considered as an approach to empirical therapy as evident from its activity, in this study, against a wide range of extensively resistant Gram-positive and Gram-negative bacteria. Also, as projected in earlier studies, Elores[TM] is not only a carbapenem-sparing option, rather it could be utilised as the sparer of last resort antibiotics, such as colistin and tigecycline, as it was found to be non-inferior to tigecycline in VRE isolates and non-inferior to colistin in Gram-negative bacterial isolates. The results of this study, especially the activity of Elores[TM] against colistin-resistant *Enterobacterales*, MRSA and VRE, warrants the need for large scale clinical trials on patients infected with these resistant organisms in order to validate its clinical efficacy against infections caused by these life-threatening bacteria.

One major limitation of our study was that we did colistin susceptibility by disc diffusion and VITEK automated method but no microbroth dilution or detection of *mcr-1* gene was done. Another limitation of our study is that the interpretation for Elores[TM] was by manufacturer and not CLSI and some groups of organisms tested are quite small, e.g. only four *A. baumannii* and nine *P. aeruginosa*. We also did not test for other common carbapenemases types prevalent in India, apart from NDM-1.

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BOOK REVIEW

Reviewed by Mike Legge

Silent Witnesses. The Often Gruesome but Always Fascinating History of Forensic Science

Author: Nigel McCrery

Publisher: Chicago Review Press, 2017

The author is an ex police officer with a special interest in forensic science. He was the creator of the award winning forensic science drama "Silent Witness" and has written a number of fiction-based crime books based on forensic analysis. In "Silent Witnesses" (sub-titled "the Gruesome but Always Fascinating History of Forensic Science") the author traces the history of the development of forensic science and analysis using a series of case studies, including brief comment (but significant) on the discovery of Sir Alec Jeffrey's that DNA could identify crime perpetrators as well as its victims. (Genetic finger printing) The book is well structured with the introduction setting the scene with the first use of DNA analysis ultimately identifying the killer of a young woman in the UK four years after the murder. The author has developed the book into techniques relating to forensic analysis, i.e. "Ballistics, Blood, Trace Evidence, The Body, Poisons and DNA. In each chapter he provides the historic background to how the development of forensic analysis shaped the use of the techniques used today. Each chapter has anecdotal case studies and importantly the people who had the ideas and developed the techniques. It also considers the controversies in the developing areas of forensic analysis and the historic difficulties juries had in understanding results and outcomes of the analysis. An underlying trend through the book are the issues relating to crime scene analysis and the many difficulties there were in getting the concept of protecting the scene of the crime as well as collecting evidence, including the origin of the well-known phrase: "every contact leaves a trace", which dates from the early 1900s. For anyone interested in the history for forensic science and analysis this is an excellent well-written book which is easy to read and understand the concepts of how modern forensics has developed and the people behind the developments.

Emergence of linezolid resistance in Enterococci: prevalent genotypes and resistance pattern in vancomycin-resistant Enterococci in a North-Indian tertiary care hospital

Hiba Sami, Anuradha Singh, Shariq Ahmed and Mohd. Shahid

ABSTRACT

Introduction: Vancomycin-resistant-Enterococci (VRE) has become a challenging nosocomial pathogen. Infections with VRE can pose therapeutic problems and are associated with increased patient morbidity and mortality worldwide. This study was primarily conducted to find out the prevalence of linezolid resistance, VRE and *vanA* gene in enterococcal isolates in a tertiary care hospital of North India.

Materials and methods: Enterococci were isolated from urine and pus clinical samples and identified to species level using standard methods. Vancomycin and linezolid susceptibility was determined by disc diffusion method. All VRE isolates were further confirmed by Vitek 2 automated system. Polymerase chain reaction (PCR) was used to detect the presence of *vanA* and *vanB* genes in VRE isolates.

Results: One thousand and fourteen (1014) isolates were identified as Enterococcus species. Thirty-one Enterococcus species were suspected as vancomycin-resistant-Enterococci (VRE) by the disc diffusion method, of which twenty eight (2.76%; 28/1014) were further confirmed as VRE by Vitek 2. Among these 31 isolates, the predominant isolate was *E. faecium* {30 (2.95%; 30/1014)} and only 1(0.09%; 1/1014) isolate was identified as *E. faecalis.* Among the 28 confirmed VRE isolates by Vitek 2, 27 (96.42%; 27/28) were flagged as *vanA* VRE isolates and 1 (3.57%; 1/28) was flagged as *vanB* VRE isolate by Vitek 2. Among the 28 confirmed VRE isolates by Vitek 2, 27 (96.42%; 27/28) were flagged as *vanA* VRE isolates and 1 (3.57%; 1/28) was flagged as *vanB* VRE isolate by Vitek 2. PCR confirmed the presence of *vanA* gene in 27 (2.66%) isolates. No *vanB* gene was detected by PCR. Prevalence of linezolid resistance was 0.88% (8/1014) in enterococcal isolates and 29% (8/28) in VRE Isolates. All the VRE isolates were sensitive to tigecycline.

Conclusion: Emergence of linezolid resistance is alarming. Furthermore, the presence of VRE and *vanA* gene in significant numbers in our clinical setting is also worrying and indicates a need to implement strict antibiotic stewardship programme to prevent the spread of resistant isolates (such as VRE and others) and losing all treatment options.

Keywords: vancomycin-resistant-Enterococci, linezolid-resistance.

INTRODUCTION

Enterococci, besides being among the members of healthy human intestinal flora, are one of the leading causes of highly antibiotic-resistant, hospital-acquired infections (1). Vancomycin-resistant-Enterococci (VRE), as hospital-associated pathogens, were first identified during the mid-1980s in Europe and have continued to spread rapidly worldwide (2). Infections with VRE can pose therapeutic problems for clinicians and an associated increase in patient morbidity and mortality worldwide (3).

Six different types of glycopeptide resistance genes have been described in enterococci: *vanA, vanB, vanC, vanD, vanE,* and *vanG* (4), most important of which are *vanA* and *vanB*. Enterococci containing the *vanA* gene are highly resistant to vancomycin and teicoplanin antibiotics, while enterococci containing the *vanB* gene show high resistance to vancomycin and susceptibility to teicoplanin (5). VRE are becoming important nosocomial pathogens (6), leaving linezolid as an alternate treatment option for these resistant strains. However, with the widespread use of linezolid, linezolid-resistant isolates are emerging too (7).

Therefore, this study was conducted to identify the species and antimicrobial resistance pattern of enterococcal isolates in our clinical setting and to find out the prevalence of linezolid resistance, vancomycin-resistant-enterococci and the prevalent glycopeptide resistance genes in our region of North India.

MATERIALS AND METHODS
Study population
The study population included patients attending the outpatient and inpatient departments of a tertiary care hospital in North India and was conducted over a period of 12 months (March 2018 to February 2019) in the Department of Microbiology, Jawaharlal Nehru Medical College Hospital, Aligarh Muslim University, Aligarh. A total of 28,140 sample of pus and urine were submitted to the Clinical Microbiology Laboratory during this period.

Isolation and identification of Enterococci
One thousand and fourteen Enterococci were isolated from urine and pus samples. The isolates were identified to species level using standard procedures (8).

Antimicrobial susceptibility testing
Antimicrobial susceptibility testing was determined for all the isolates using the Kirby-Bauer disc diffusion method, as recommended by Clinical and Laboratory Standards Institute (CLSI) M100-S27 (9). The antimicrobials tested for Enterococci were: vancomycin (30µg), teicoplanin (30 µg), amoxicillin (30 µg), levofloxacin (5µg), azithromycin (15 µg), linezolid (30 µg), and nitrofurantoin (300 µg). All VRE strains were confirmed by Vitek 2 (BioMerieux, USA).

Polymerase chain reaction assay for *vanA* and *vanB* genes

The PCR amplification of *van* genes were carried out as per standard protocol (10). PCR was performed in 25 µl volumes that contained 20-200 ng DNA, 0.5 µM of specific primers for *E. faecalis* (ddIE1:ATCAAGTACAGTTAGTCTTTATTAG, ddIE2:ACG ATTCAAAGCTAACTGAAT, *E. faecium* (ddIF1:TTGAGGCAGACCAGATTGACG,ddIF2:TATGAC GCGACTCCGAT), *vanA* (vAF: ATACTGTTTGGGGGGTTGCTC, vAR: TTTTTCCGGCTCGACTTCCT) and for *vanB* (vBF: G C G G G G A G G A T G G T G C G A T A C A G , v B R : GGAAGATACCGTGGCTCAAAC) with 1.5 mM MgCl₂, 200 µM of each dNTP, 1X PCR buffer and 2 U DNA polymerase.

PCR conditions were as follows: an initial denaturation of 10 min at 94°C, followed by 35 cycles of denaturation at 94°C (1 min), annealing at 58°C for 1 min and extension at 72°C for 1 min, followed by a final extention at 72°C for 10 min.

Negative controls consisted of the PCR components of the reaction mixtures lacking enterococci DNA. PCR products were electrophoresed in 1.5% agarose gels and after staining with 0.5µg/ ml ethidium bromide, visualised under UV light. The size of the fragments was determined by comparing with O'RangeRuler 100 bp DNA Ladder (Thermo Fisher Scientific).

RESULTS

A total 16,229 urine samples were received in the lab, of which 1,522 (9.37%) showed growth of Gram-positive bacteria. Of these culture positive urine samples, 699 (45.9%) had growth of Enterococcus Species.

A total 1,1911 pus samples were received in the bacteriology lab during the study period, 2,323 (19.5%) had growth of Gram-positive bacteria, among which 315 (13.56%) were Enterococcus species.

Out of the total of 1,014 Enterococcal isolates, 31 (3.05%) were resistant to vancomycin. Among these 31 isolates, the predominant isolate was *E. faecium* [30 (2.95%; 30/1014)] and only one (0.09%; 1/1,014) isolate was identified as *E. faecalis* by biochemical tests and further confirmed by Vitek 2. PCR for species identification showed concordant results in all the 31 isolates confirming the results of Vitek 2 (Figure 1).

Antibiotic resistance pattern

All 31 VRE were confirmed by Vitek 2, which confirmed vancomycin resistance in 28 of the 31 samples. However, three samples showed discordant results as they were vancomycin resistant by disc diffusion and vancomycin sensitive by Vitek 2. These samples failed to show any band in PCR for *vanA* or *vanB*. The pattern of antibiotic resistance in isolates is summarised in Figure 2.
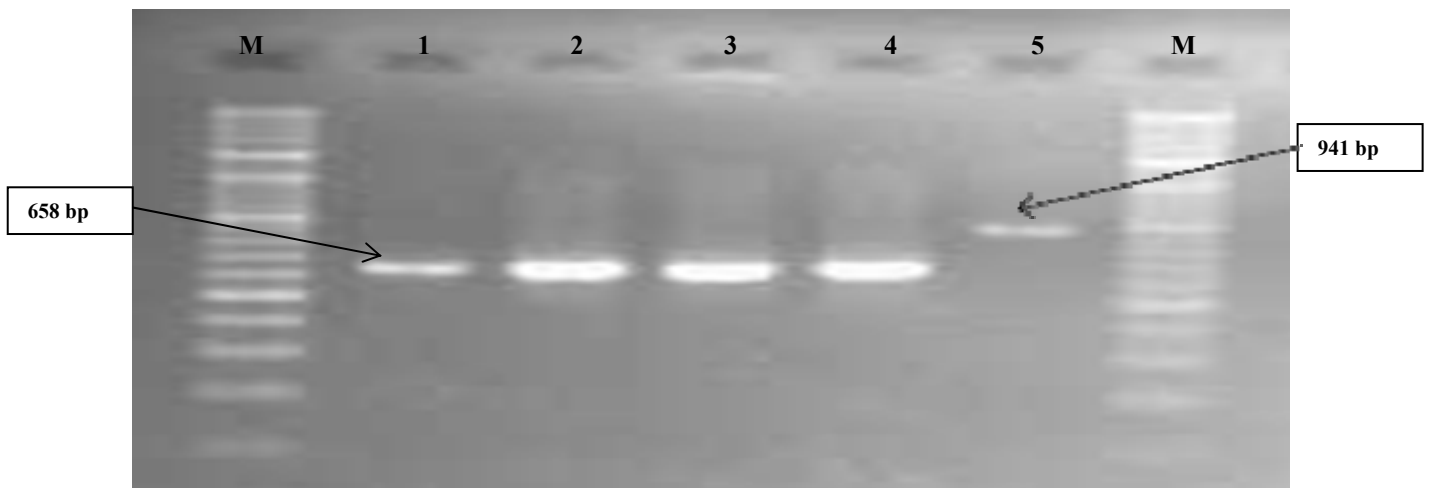

Figure 1. Agarose gel electrophoresis of VRE isolates for species identification, Lane M:100 bp DNA ladder; Lane 1-4 *Enterococcus faecium* (658bp) and Lane 5 *Enterococcus faecalis* (941bp).
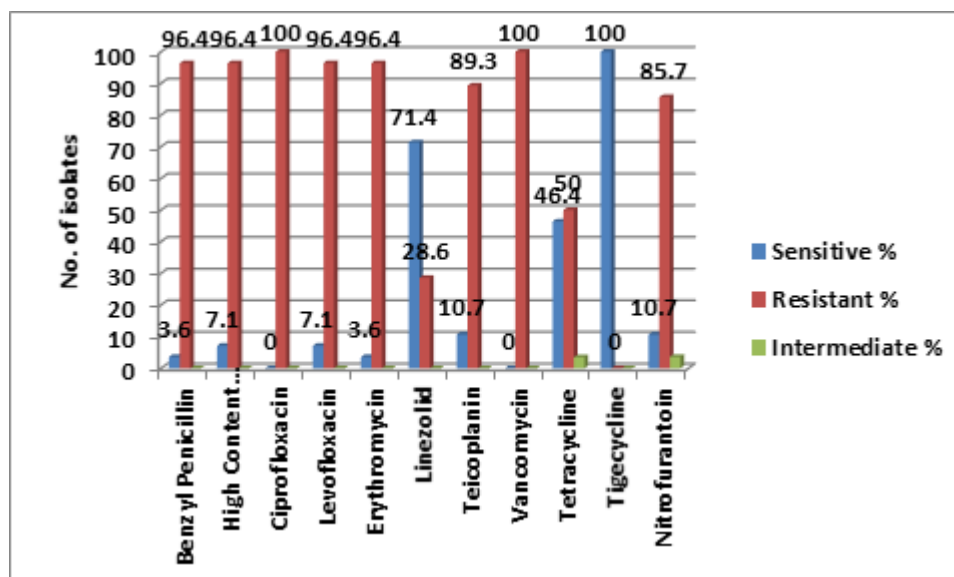

Figure 2. Antibiotic sensitivity profile of all VRE isolates characterized by Vitek 2 automated system (n=28).

The result show that the majority of VRE isolates were resistant to high content gentamycin (92.8%), benzyl penicillin (96.4%), ciprofloxacin (100%), levofloxacin (92.8%), erythromycin (96.4%) and nitrofurantoin (85.7%) while 29%, 50% and 89% of VRE isolates were resistant to linezolid, tetracyclines and teicoplanin respectively. All of the VRE isolates were sensitive to tigecycline.

Detection of *van* genotypes

Vitek 2 detected *vanA* genotypes in 27 VRE isolates, which were further confirmed by PCR and a 734bp PCR product was obtained for *vanA* (Figure 3). However, one sample which was flagged to have *vanB* -like mechanism of resistance in Vitek 2 could not be confirmed by PCR, this isolate was negative for *vanA* also. All the isolates in which *vanA* were detected were *E. faecium*.
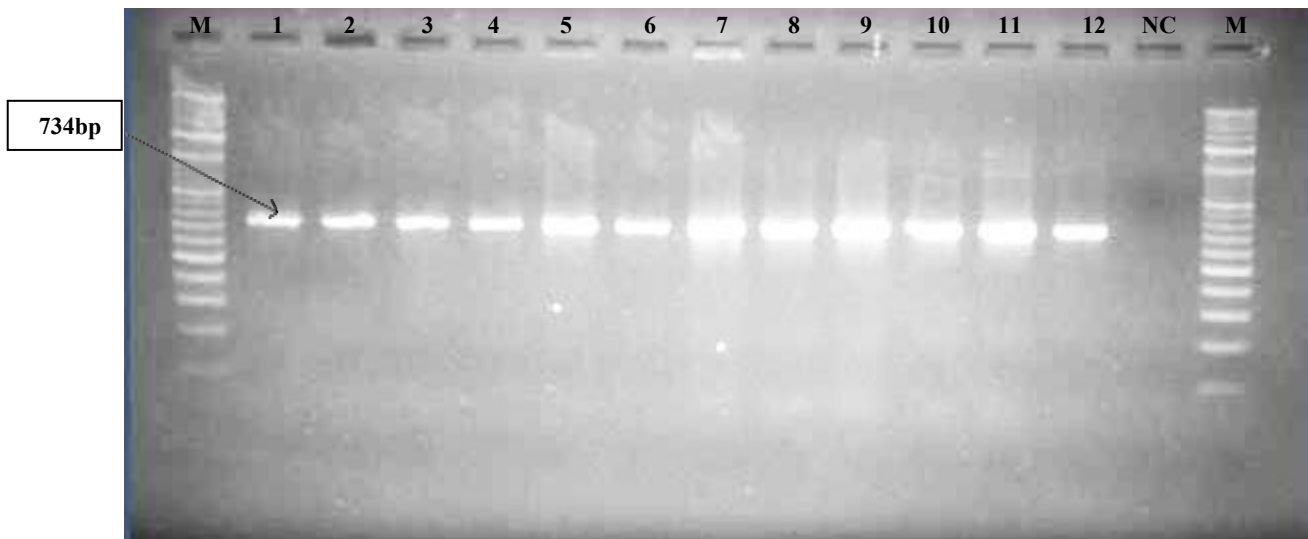

Figure 3. Agarose gel electrophoresis of VRE isolates (PCR product of 734 bp indicating *vanA* gene). Lane M:100 bp DNA ladder; lane NC: Negative Control; lanes 1-12: *vanA* positive samples.

DISCUSSION

Recent years have seen a tremendous increase in the number of patients who are colonised with VRE (6). Resistance to vancomycin can be important in infections caused by enterococcal species, as vancomycin is a last line of antibiotic therapy in many patients. In addition, vancomycin resistance genes can be transmitted from enterococcus to staphylococcus through plasmids, potentially causing serious life-threatening infections (11). Antibiotic stewardship programmes for antibiotic usage, especially for last option antibiotics, can prevent the spread of resistant isolates and the potential for losing all treatment options in the near future.

We investigated the prevalence of VRE in clinical isolates at our institution by phenotypic and genotypic methods, finding *Enterococcus faecium* as the most common VRE. Other studies from India have also reported the predominant prevalence of *E. faecium* (12, 13). The prevalence of VRE in our study was 2.76% (28/1014) which concurs with other studies from India that report the prevalence of VRE to be from 1 to 8.7 % (14,15).

Our study found difference in results when done by disc diffusion and Vitek 2. Several studies have found differences between automatic and classical or molecular systems (16). We suggest the use of VITEK 2 automated system to confirm the findings of disc diffusion method in order to prevent the potential over-reporting of VRE.

According to the antibiogram, all *E. faecalis* isolates were susceptible to tigecycline. A reason for high susceptibility to this antibiotic is probably its less prescription by physicians. Kafil et al from Iran (10) showed that VRE isolates were susceptible to linezolid and nitrofurantoin, which is in contrast to our study as 29% and 85% of our VRE isolates were resistant to linezolid and nitrofurantoin respectively. Linezolid non-susceptible enterococci isolates are an emerging clinical problem (17). Healthcare institutions should be on the alert for the risk of clonal spread of linezolid-resistant-enterococci. Whilst VRE generally responds well to linezolid, linezolid-resistance may develop as a consequence of prolonged linezolid exposure (18).

Cho et al from South Korea reported the prevalence of 1.8% linezolid resistance in VRE isolates (19). In our study, we found the prevalence of linezolid resistance to be 0.78% (8/1014) in enterococcal isolates and 8/28 (29%) in VRE isolates. To the best of our knowledge based on an internet search, from India, almost all *Staphylococcus* species and *Enterococcus* species remain linezolid sensitive, except for occasional case reports (20). This study demonstrates the increasing linezolid resistance in North India and urges a need to take action so as to emphasise rational use of antibiotics and to keep linezolid as a reserve agent. Few of our *vanA* isolates were teicoplanin sensitive but these types of discrepancies have also been reported in earlier studies (21-23).

Enterococcus faecium is a commonly encountered opportunistic pathogen that is most commonly found as a commensal of the human and animal gut but can also survive in the environment. *E. faecium* is notorious in acquiring resistance genes that, when expressed, can effectively circumvent most of the antimicrobials. (24). In this study, PCR proved helpful in detecting the *van* genotypes present in this geographic region. The majority (96%) of our isolates had *vanA* genotype. Compared with *vanB*, the *vanA* gene is known to have increased transferability (25), which may explain the higher prevalence of *vanA* isolates. Furthermore, the *vanB* gene is mainly associated with epidemics and food contamination, while *vanA* gene is associated with clinical strains (26). Another study from North India has reported the prevalence of 90.5% of *vanA* genotypes in VRE which in concordance with our study (14). On the contrary, a study from North East India has reported the prevalence of 56.25 per cent of *vanA* genotypes in VRE (27). In our study, one sample which was flagged to have *vanB* like mechanism of resistance in Vitek 2 could not be confirmed by PCR, this isolate was negative for *vanA* also. As we have not tested other *van* genotypes there is a possibility that other *van* genes might be present in this strain which could not be amplified.

One major limitation of our study is that we didn't test for linezolid resistance in vancomycin susceptible isolates as linezolid resistance can occur in vancomycin susceptible enterococci also.

In conclusion, the occurrence of VRE and the presence of *vanA* gene, in a significant percentage in our clinical setting, is alarming and thus suggests the need to implement a strict antibiotic stewardship programme in order to prevent the spread of resistant isolates (such as VRE and others) and potentially losing all treatment options. Through the detection of resistant strains and proper treatment of infected patients, resistance development to vancomycin can be prevented. Although linezolid remains highly active against Enterococcus, emergence of linezolid-resistant-Enterococci is alarming as it further limits the therapeutic options. Our study showed that though linezolid and tigecycline are the most effective drug for VRE strains, resistance to linezolid is emerging and should be prescribed only in reserve cases.

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Clinical evaluation of a newly developed point-of-care haemoglobinometer. Comparison with colorimetric and cell counter haemoglobin determination methods

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ABSTRACT

Context. Haemoglobin (Hb) determination is considered the first test in assessing anaemia. Many methods have been used to estimate Hb values, each with its own specific principles and criteria. Recently, a new point-of-care (POCT) device (URIT-12 haemoglobinometer) has been developed.

Objective. We compared the results obtained by URIT-12 to that of cyanmethemoglobin and cell counter analyser methods, in order to establish the reliability of using URIT-12 hemoglobinometer in estimating Hb values of anaemic patients.

Study design. Hb levels of 50 outpatients were prospectively screened by three methods: URIT-12 Haemoglobinometer, cyanmethemoglobin and gold-standard cell counter analyser. Measurements of URIT-12 and cyanmethemoglobin were performed in parallel and in duplicate. A cut-off Hb value of 120.0 g/L (females) and 130.0 g/L (males) was used for assessing anaemia.

Results. A total of 50 outpatients were analysed; [(26 males (52%) and 24 females (48%)]. URIT-12 haemoglobinometer was less precise for its duplicates comparing to cyanmethemoglobin method, (CV 0.2 and 0.18) respectively. URIT-12 hemoglobinometer overestimated Hb values (mean bias, 10.78 g/L; 95% limits of agreement, 8.576-12.983) compared to the cyanmethemoglobin method (mean bias, 6.496 g/L; 95% limits of agreement, 4.832-8.159). The URIT-12 method revealed false high Hb values in 48.6% and the cyanmethemoglobin revealed false high Hb values in 21.6% of patients compared to the cell counter method. URIT-12 method was less sensitive; 51.4% and positive predictive value was 67.3%. Both methods were perfectly specific to diagnose non-anaemic patients.

Conclusion. The URIT-12 haemoglobinometer is not suitable as an alternative for the traditional cyanmethemoglobin method or the standard cell counter analyser in evaluating Hb values in anaemic patients.

Keywords: haemoglobin, URIT-12 haemoglobinometer, cyanmethemoglobin, anaemia.

N Z J Med Lab Sci 2020; 74: 31-34

INTRODUCTION

Haemoglobin (Hb) determinations are considered to be the most frequent performed test by health care units in assessing anaemia (1). Many haemoglobin determination methods have been developed, from the colorimetric methods to point of care testing (POCT). These methods have been very attractive for researchers who try to point out the performance of each method, focusing on the advantages, disadvantages, accuracy, precision, specificity and sensitivity in comparison to well accepted reference methods such as the cell counter, which is still considered the most acceptable one (2). Photometric detection of cyanmethemoglobin, which is known for its stability as a Hb-derived compound, is still the most-used method for determining Hb levels (1). This might be due to the availability of ready to use reagents in addition to cyanmethemoglobin standard solutions for calibration (3). In this method, haemoglobin is converted to cyanmethemoglobin after the addition of potassium cyanide and ferricyanide and the absorbance is measured at 540 nm by a spectrophotometer against a standard solution (4). However, POCTs in haematology have been used as a replacement to the traditional methods throughout the world as the need to minimise the turnaround time of test results coupled with increased accuracy (5). The use of POCT devices to determine Hb concentration would replace the traditional methods in many health care areas, especially when a rapid Hb determination related to urgent therapeutic intervention is required (2).

The Hemocue haemoglobinometer is a well-known POCT device used to measure Hb with a principle depending on converting haemoglobin to azidemethaemoglobin and the transmitted light absorbance is measured at 565nm and 800nm where absorbance is proportional to the haemoglobin concentration (6). A new POCT haemoglobinometer, the URIT-12 (URIT Medical Electronics Co., Ltd, Guangxi, China) has been developed which measures the optical reflectance to determine haemoglobin concentrations. The URIT-12 uses strips that utilise a dry chemistry colorimetric method (Azido methemoglobin method). After applying the blood drop to the test spot on the strip, blood disperses immediately within the membrane and contacts the reagent, with the reaction product absorbing spectrum in the range of 500nm to 600nm. The change in the membrane reflectance is detected by the meter's optical detectors where the membrane reflectance is inversely proportional to the haemoglobin concentration. The range of measured Hb as revealed by the manufacturer is from 40.0 to 240.0 g/L where results under 40.0 g/L and over 240.0 g/L are displayed as "Lo" and "Hi", respectively.

The aim of this study was to evaluate the URIT-12 haemoglobinometer and a cyanmethemoglobin method against a cell counter method, to ascertain the URIT-12 haemoglobinometer utility for the the initial diagnosis of anaemia and whether it could replace the traditional cyanmethemoglobin method.

MATERIAL AND METHODS

This prospective observational study was approved by the ethics committee at our institute. EDTA venous blood samples were collected from 50 outpatients (26 male and 24 females) visiting a private central clinical laboratory, with Hb values ranging from 45.0-150.0 g/L to allow a wide range of Hb levels. We followed the normal reference ranges adopted by the World Health Organization where the Hb cut-off for patients who are considered anaemic is <130.0 g/L for adult males and < 120.0 g/L for non-pregnant adult females (7). The blood samples were analysed for reference Hb values using a calibrated laboratory haematology analyser, the Diagon D-cell 60 (Diagon Ltd., Budapest, Hungary) according to the manufacturer's instructions. Hb analysis by the URIT-12 haemoglobinometer and by the cyanmethhemoglobin method were done in duplicate using the same venous blood samples and all tests were run at room temperature within 24 hours of blood collection.

For measurement with the URIT-12 haemoglobinometer, 15µL of venous blood was taken and added immediately to the strip, where the Hb reading was available after about 10-15 seconds. For measurement with the cyanmethhemoglobin method, 10µL of venous blood was added to 2.5 mL of ready-to-use Drabkins reagent (SPECTRUM, Cairo. Egypt) and incubated at room temperature for 5 minutes then read by a spectrophotometer at 540nm where the Hb value in g/L was calculated after multiplying the result by the factor of 36.77 according to the manufacturer's instructions. With respect to the Hb value obtained by cell counter analyser (reference method) accuracy, precision, sensitivity, specificity, positive and negative predictive values (PPV and NPV) of venous Hb measurements with the URIT-12 hemoglobinometer and cyanmethemoglobin method for the diagnosis of anaemia were determined.

Statistical analysis

The mean, maximum and minimum values, 95% confidence interval upper and lower limits were compared for the three Hb methods. Coefficient of variation (CV) was used to evaluate the within-run precision of the duplicate measurements. A paired sample-t test was used firstly to compare the duplicate measurements of each method. Then it was used to compare the two methods to the reference method, respectively. Bland-Altman analysis was used to check the accuracy of the two methods comparing to the reference method. Sensitivity, specificity, negative predictive value and positive predictive values of cyanmethemoglobin and URIT-12 method were evaluated in comparing to the cell counter reference method. Sensitivity was defined as the ability of the method to correctly identify anaemic patient who has a low Hb value (less than cut-off) among all patients with a low Hb values in the reference method. Specificity is ability of the method to correctly identify non-anaemic patient who has an acceptable Hb value (greater than cut-off) among all patients with an acceptable Hb in the reference method. Negative predictive value is the percentage of non-anaemic patients with a negative test (Hb>cut-off) who had an acceptable Hb value (Hb>cut-off in the reference method). Positive predictive value is the percentage of anaemic patients with a positive test (Hb<cut-off) who truly have a low Hb value (Hb<cut-off) in the reference method. Statistical significance was set at the p 0.05 level. Microsoft excel (Professional plus 2013) and SPSS 24 (IBM®, SPSS®, statistics, version 24) were used for statistical analysis.

RESULTS

Fifty outpatients' venous blood samples [(26 males (52%) and 24 females (48%)] were taken to compare Hb measurements by the three methods (cyanmethemoglobin, URIT-12 hemoglobinometer and cell counter). The initial analysis of the measurements obtained by the three methods revealed significant differences. Differences between the duplicate measurements for each method were not significant with a mean difference of 0.176 g/L (95% limits of agreement: -1.568-1.92) for the cyanmethemoglobin method whereas the mean difference was 0.16 g/L (95% limits of agreements: -0.967-1.287) for the URIT-12 Haemoglobinometer. Therefore, with the exception of within-run precision, all further comparisons were performed depending on the first value obtained. Within-run precision of duplicates for the cyanmethemoglobin method was more precise with a CV of 0.18% for its duplicate measurements whereas the CV of the URIT-12 hemoglobinometer was 0.2% (Table 1).

In the Bland-Altman analysis a bias of 6.496 g/L was found when comparing the cyanmethemoglobin method to the cell counter method (95% limits of agreement: 4.832-8.159), while a bias of 10.78 g/L was found when comparing the URIT-12 method to the cell counter method (95% limits of agreement: 8.576-12.983) (Figure 1). Thus, both methods systematically overestimated Hb values. Table 2 summarises sensitivity, specificity, NPV and PPV.

Table 1. Results of duplicate measurements*

Measurements	Cyanmethemoglobin		URIT-12 hemoglobinometer		Cell counter**
	Read 1	Read 2	Read 1	Read 2	
Mean Hb (g/L)	115.6	115.5	119.9	119.8	109.1
95% CI	109.9-121.3	109.5-121.4	112.9-126.2	112.4-126.3	103.3
Maximum	164.7	162.2	166.0	166.0	150.0
Minimum	46	45.6	42.0	40.0	45.0
SD	20.9	21.4	23.9	25.2	20.0
Coefficient of variation (CV)	0.18	0.18	0.19	0.21	/

* Difference between read 1 and read 2 for each method was not significant (p>0.05), while differences between cyanmethemoglobin, URIT-12 and cell counter methods were all statistically significant (p<0.05).
**One read only was used in the cell counter method.

Table 2. Performance of the cyanmethemoglobin and URIT-12 methods in comparison to the cell counter reference method[*].

Method	Sensitivity %	Specificity %	Negative predictive value (NPV)	Positive predictive value (PPV)
Cyanmethhemoglobin				
All	78.4	100	100	82.2
Male	66.7	100	100	75.2
Female	86.4	100	100	88.0
URIT-12 method				
All	51.4	100	100	67.3
Male	46.7	100	100	65.2
Female	54.5	100	100	68.7

[*]Sensitivity is the ability of a method to correctly identify anaemic patient who has a low Hb value (less than cut-off) among all patients with a low Hb values in the reference method. Specificity is ability of method to correctly identify non-anaemic patient who has an acceptable Hb value (greater than cut-off) among all patients with an acceptable Hb in the reference method. Negative predictive value is the percentage of non-anaemic patients with a negative test (Hb>cut-off) who truly have an acceptable Hb value (Hb>cut-off in the reference method). Positive predictive value is the percentage of anaemic patients with a positive test (Hb<cut-off) who truly have a low Hb value (Hb<cut-off) in the reference method.

Regarding sensitivity, of 50 patients, 29 (78.4%) were correctly identified with low Hb values in the cell counter method using cyanmethemoglobin method which made it more sensitive than the URIT-12 method where 19 (51.4%) were identified with a low Hb. The URIT-12 method had more falsely high Hb values (18; 48.6%) comparing to (8; 21.6%) of the cyanmethemoglobin method. However, both the cyanmethemoglobin and URIT-12 hemoglobinometer methods were 100% specific to correctly evaluate a patient who was non-anaemic among all non-anaemic patients (Hb>cut-off). NPV showed that both methods correctly identified patients as non-anaemic and were truly identified as non-anaemic (Hb>cut-off) with the cell counter method. However, PPV showed that the cyanmethemoglobin method was more reliable regarding detection of anaemic patients with Hb<cut-off who were truly diagnosed with a low Hb value (Hb<cut-off) in the cell counter reference method (82.2%) in comparison to the URIT-12 method (67.3%).
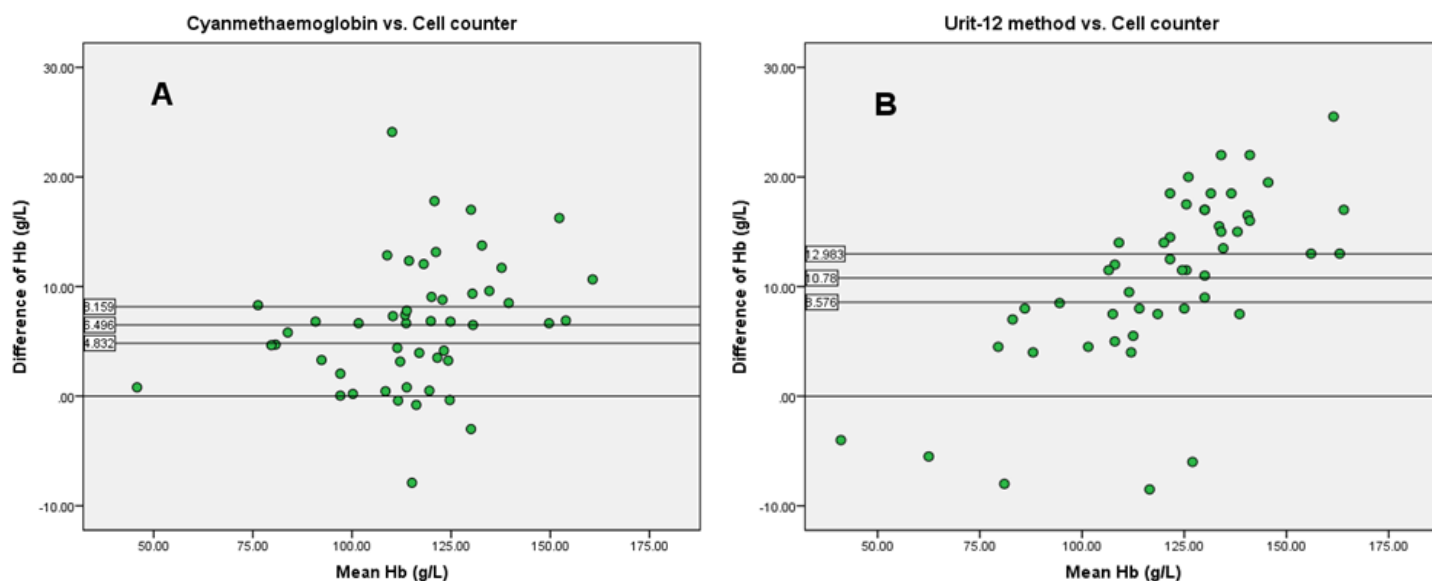

Figure1. Bland-Altman analysis with mean difference and ±13.6 standard deviations as 95% limits of agreement. The cyanmethemoglobin test overestimates Hb values (A) as well as for the URIT-12 hemoglobinometer (B).

DISCUSSION

This study prospectively examined the utility of using a newly developed haemoglobinometer (URIT-12) to estimate Hb values. Only venous blood samples were tested as different blood sources could lead to variations due to anatomical and technical reasons (8). Both the URIT-12 and cyanmethhemoglobin methods show an overestimation of Hb values compared to the the cell counter. Within-run precision showed that the cyanmethhemoglobin method was more precise (CV: 0.18%). Moreover, the cyanmethhemoglobin method was more sensitive (78.4%). Both methods were specific (100%) for determining non-anaemic patients with Hb values>cut off (Tables 1 and 2).

When comparing our results of using this new type of POCT in this study to the results obtained by other studies using different POCTs, such as the Hemocue hemoglobinometer, the latter showed acceptable accuracy when compared to the cell counter method (6). However, another study showed that the mean of Hb value obtained by the Hemocue (147 ± 14.9 g/L) was higher by 0.24 g/L when compared with the cell counter reference Hb values (138 ± 15.2 g/L) (9). Thus, some concerns regarding accuracy and reliability of the Hemocue traditional method also have been raised recently which has urged others to examine the utility of other POCT devices, such as the Hemocue ® gravity method, which showed more accuracy, despite the high cost and limited disposable cuvettes (10).

In our study, and although appropriate from the perspective of simplicity, test time consuming, less specimen handling requirements of using URIT-12 hemoglobinometer, results obtained were not quite encouraging to use this new POCT device as an alternative to the cell counter method or even the traditional cyanmethemoglobin method. Our study had some limitations such as the lack of data from other studies of the URIT-12 haemoglobinometer as that would be quite critical for comparing our data. Other limitations include the lack of interference studies, within/between run precision, the small sample size of only 50 patients and, because of cost, using single reading from the cell counter.

In summary, we showed that the URIT-12 hemoglobinometer overestimated Hb values with more false high Hb (18 (48.6%)) which made it unreliable for evaluating Hb values of anaemic patients and should not replace Hb estimation by cell counters, which is still considered the gold standard.

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ANNOUNCEMENT

Election of Rob Siebers as Vice-President of the World Association of Medical Editors

Rob Siebers, Editor of the New Zealand Journal of Medical Laboratory Science, has been elected Vice-President of the World Association of Medical Editors (WAME) for a two year period (2020-2021) and is President-elect for 2022-2023. Rob has previously served three terms as Board Director, is currently Chair of its Small Journal Task Force and has been a Member of a number of its committees in the past.

WAME was established in 1995 as a voluntary association of editors of peer-reviewed medical and biomedical journals.

Currently it represents over 1,800 members of over 1,000 journals from 92 countries. WAME's goals are to facilitate worldwide cooperation and communication among editors, improve editorial standards, promote professionalism and encourage research on principles and practice.

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Is serum procalcitonin a reliable test for the detection of acute appendicitis?

*Ali Khavanin , Mohammadreza Maleki Verki , Mohammad Ali Fahimi ,
Ali Delirrooyfard , Ramazanali Hajizadeh and Pedram Nazari*

ABSTRACT

Background: Acute appendicitis is the most important cause of referral of patients with acute abdominal pain to the emergency department (ED). According to research on the value of procalcitonin (PCT) on the diagnosis of appendicitis, the present study aimed to investigate the serum PCT level and its diagnostic accuracy in acute appendicitis.

Methods: The current prospective study was conducted on healthy individuals and patients suspected of acute appendicitis admitted to the ED of Imam Khomeini and Golestan Hospitals, Ahvaz, Iran. The inclusion criteria were being healthy or suspected of having appendicitis, aged above than 17 years, while the exclusion criteria were pregnant women, a history of appendectomy, inflammatory diseases, active cancers or abdominal trauma. PCT was measured by ELISA, and white cell count (WBC) and % polymorhonuclear neutrophils (PMN) with a haematology analyser.

Results: Eighty-six subjects (43 cases and 43 controls) participated, of which 60 were male. The mean age of the subjects was 34.4 ± 10.5 years. The results showed that the sensitivity of PCT (cutoff point: 0.11 ng/mL), WBC (cutoff point: 10 x 10^9/L), and % PMN (cutoff point: 65%) for the diagnosis of acute appendicitis was 90.7%, 79.1%, and 90.7% respectively, while their specificity was 48.8%, 86.0%, and 90.7% respectively. The diagnostic accuracy of PCT, WBC, and % PMN was 69.8%, 82.6%, and 90.7%, respectively.

Conclusions: Although PCT seems to be a high-sensitivity test for the diagnosis of acute appendicitis, because of its low specificity the use of other criteria, especially % PMN, seems to be necessary in this regard.

Keywords: Appendicitis, procalcitonin, diagnostic accuracy, % polymorhonuclear neutrophils, white cell count.

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INTRODUCTION

Acute appendicitis is one of the most common causes of referral of patients with acute abdominal pain to the emergency department (ED) (1). It is reported that up to 7% of people develop acute appendicitis during their life, which occurs more often within the age range of 10 to 30 years (2). Despite the advances made in the diagnostic tools, the diagnosis of acute appendicitis is still an important challenge for the ED and the rate of negative appendectomy varies between 8%-12% in males and 25-45% in females (3-5).

Currently, the most important diagnostic criteria for appendicitis is reliance on clinical manifestations and laboratory tests, such as the white cell count and their differentiation (6,7). The timely and precise diagnosis of appendicitis is necessary to reduce the risk of appendiceal rupture. Negative appendectomy is associated with surgery and anesthesia complications, similar to those of positive appendectomy, including post-operative infections, intestinal obstruction due to adhesion, and the risk of infertility in young women. Therefore, many methods are proposed to improve diagnostic accuracy; ie, laboratory tests, sonography, CT scan, and laparoscopy (2). One of the most applicable criteria for the diagnosis of appendicitis is the Alvarado score that, despite all its diagnostic benefits, it also has many drawbacks (8-11), including the fact that the Alvarado scoring system is structurally relied on the review of patients with suspected appendicitis who had undergone surgery. Also, biomarkers are not involved in the Alvarado scoring system. This is why the results of most studies conducted in this field address the crucial role of biomarkers in the diagnosis of appendicitis (12). One of these relatively widely studied biomarker candidates is the procalcitonin (PCT). PCT is a precursor to the calcitonin hormone, secreted by the C-cells from the thyroid gland.

PCT is one of the most reliable parameters in the early detection and distinction of bacterial infections from viral ones in patients admitted to emergency wards for acute reasons, such as burns, inflammations, surgeries, respiratory problems and infantile infections (13-15). If the serum level of PCT in the first 2-24 hours of manifestation exceeds more than 0.5 ng/mL, it represents a bacterial infection and, if it increases after six hours, it is an appropriate and reliable biomarker of septic shock in the patient (16-20). Interestingly, some studies suggest that PCT has a higher sensitivity and specificity than C-reactive protein (CRP (24-21). Given the potential value of PCT as a candidate in the diagnosis of acute appendicitis, as well as controversy in some studies on the diagnostic accuracy of this biomarker, and the limitation of access to imaging facilities in some medical centers, we designed the current study aimed at evaluating the diagnostic accuracy of PCT in acute appendicitis.

METHODS

This prospective study was performed on 86 healthy and patient subjects (n=43 in each group) referred to the ED of Imam Khomeini and Golestan Hospitals in Ahvaz, Southwest of Iran from March 2017 to March 2018. Imam Khomeini and Golestan Hospitals are the largest referral centers of level 3 in southwestern Iran. Participants were selected using a sequential non-probability sampling method. The control group was selected from healthy subjects referred to the same hospital and the case group was selected from patients with suspected appendicitis, confirmed by pathologic evidence based on histopathological findings).

Inclusion criteria included healthy subjects and patients with complaints of acute abdominal pain suspected of appendicitis, all aged 17 years or above. The exclusion criteria included pregnant women, past history of appendicitis and appendectomy, inflammatory diseases, active cancers, abdominal trauma, surgery or invasive abdominal procedures within the past seven days, corticosteroids in the past 14 days, chemotherapy or immunosuppressive drugs within the past 29 days, and patients with thyroid problems (hypothyroidism, hyperthyroidism or autoimmune thyroid disease). The study

protocol was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. Written informed consent was obtained from all subjects participating in the study. All participants were assessed by an emergency specialist in terms of eligibility. All patients were routinely evaluated according to standard guidelines.

At the beginning of the study, serum samples were taken from all subjects were to measure the PCT level using the Human Procalcitonin ELISA kit (Shanghai Korain Biotech Co, China) as well as WBC and % PMN (Sysmex XN-L Cell Counter, U.S.). Patients with a high probability of acute appendicitis underwent appendectomy, under the supervision of a surgeon. Patients whose acute appendicitis was confirmed based on histopathologic findings were considered as definite cases of appendicitis and enrolled in the case group.

Statistical analysis

Descriptive statistics, including frequency distribution tables, figures, and central indices as well as normal distributions were used to interpret the variables studied. Normality of quantitative data was investigated using the Kolmogorov-Smirnov test. The relationship between quantitative variables was evaluated using t-test (independent and paired samples) and to evaluate the relationship between qualitative variables Chi-square and Pearson or Spearman correlation coefficients were used. In order to detect the sensitivity and specificity of the diagnostic tests, the ROC curve was used to determine the cutoff point. $P < 0.05$ was considered as the level of statistical significance. Data analysis was performed using SPSS software version 16.

RESULTS

A total of 86 subjects participated in the study, of which 60 (69.8%) were male. The mean age of the subjects was 34.4±10.5 years. Demographic data showed that both control and case groups (n= 43 in each group) were matched for age and gender (P >0.05). The mean PCT, WBC and % PMN in the case and control groups are shown in Table 1.

The results of the study showed that the sensitivity and specificity of the PCT test for detecting acute appendicitis at a cutoff point of 0.5 ng/mL were 18.6% and 83.7%, respectively. The positive and negative predictive values of this test were 53.3% and 50.7%, respectively. According to the ROC curve, the appropriate cutoff point for PCT was 0.11 ng/mL while its sensitivity and specificity were 90.7% and 48.8%, respectively. The positive and negative predictive values of PCT were 63.93% and 84.00%, respectively. The AUC was 0.631 and diagnostic accuracy was 69.8%. The sensitivity and specificity of WBC to detect acute appendicitis at the cutoff point of 10 x 10^9/L were 79.1% and 86.0%, respectively. In addition, the positive and negative predictive values were 85.0% and 80.4%, respectively. The diagnostic accuracy of WBC test was 82.6%. The sensitivity and specificity of % PMN in detecting acute appendicitis at a cutoff point of 65% were 90.7% and 90.7%, respectively, while the positive and negative predictive values were 90.7% and 90.7%, respectively. The diagnostic accuracy of % PMN test was 90.69% (Figure 1 and Table 2).

Table 1. Demographic data of participants.

Variables	Cases	Controls	P
Age (years), Mean± SD (Range)	35.6±8.0 (18-57)	38.3±11.4 (23-63)	0.20
Gender			
Male, N (%)	32 (74.2)	28 (65.1)	0.48
Female, N (%)	11 (25.8)	15 (34.9)	
Laboratory parameters			
Procalcitonin (ng/mL), Mean± SD	0.37±0.05	0.27±0.04	0.23
WBC x 10^9/L, Mean± SD	13.5±4.0	7.7±2.7	<0.001
%PMN, Mean± SD	81.0±12.0	40.8±19.6	<0.001

Table 2. Diagnostic accuracy of procalcitonin (ng/dl), WBC count, %PMN in acute appendicitis

Variables	Cut-off	Sensitivity (95% CI[*])	Specificity (95% CI)	Accuracy	AUC (95% CI)
Procalcitonin	0.11 ng/ml	90.7 (76.9-97.0)	48.8 (33.6-64.3)	69.77	0.631 (0.507-0.77)
WBC count	10 x 10^9/L	79.1 (63.5-89.4)	86.0 (71.4-94.2)	82.56	0.885 .(0.811-0.960)
PMN	65%	90.7 (76.9-97.0)	90.7 (76.9-97.0)	90.69	0.954 (0.911-0.990)

[*]CI: Confidence Interval.
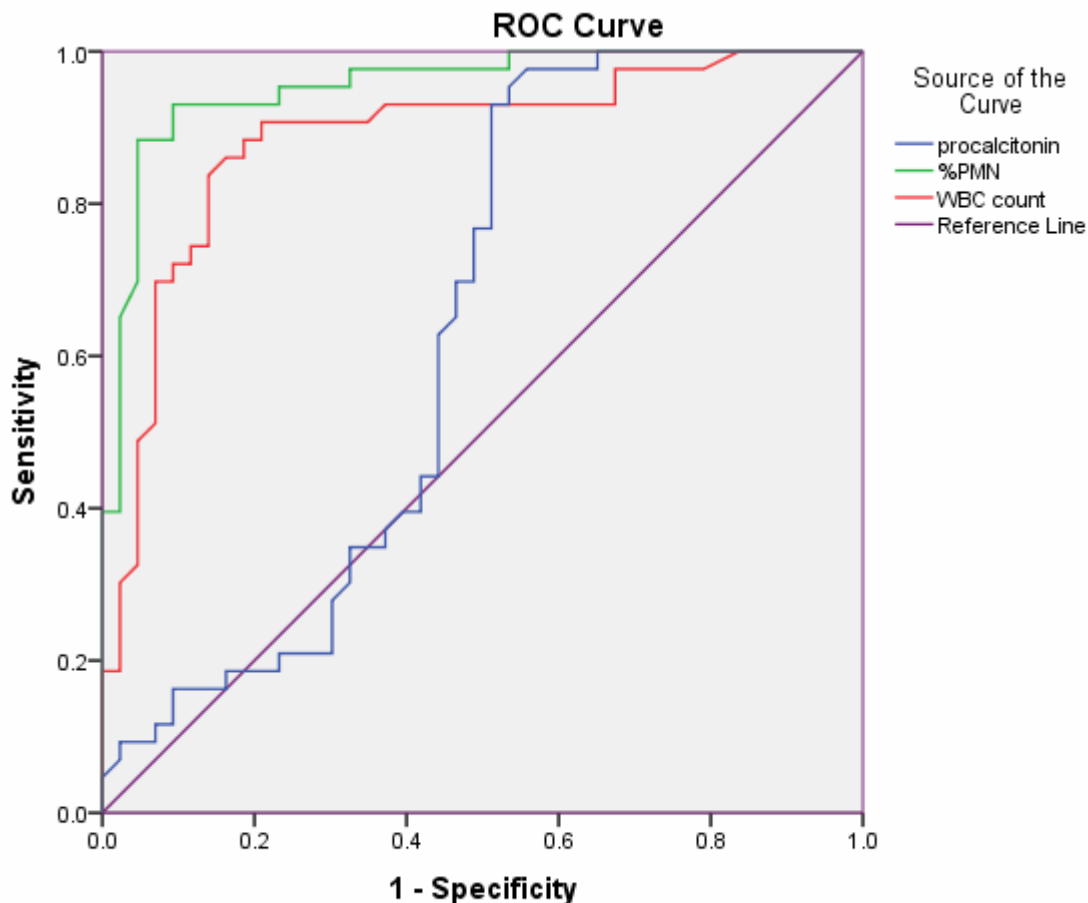
Figure 1. The area under the curve (AUC) of procalcitonin, WBC count, %PMN in acute appendicitis detection.

DISCUSSION

The results of the study showed that the sensitivity and specificity of PCT to detect acute appendicitis at the cutoff point of 0.5 ng/mL were 18.6% and 83.7%, respectively. In addition, its positive and negative predictive values were 53.3% and 50.7%, respectively. The appropriate cutoff point for PCT was 0.11 ng/mL, at which the sensitivity and specificity of the test were 90.7% and 48.8%, respectively. Khan et al., in a pilot prospective cohort study, showed that the PCT at a cutoff point of 0.39 ng/mL had a sensitivity and specificity of 25% and 92%, respectively. They showed that all patients with a PCT<0.39 ng/mL and a WBC<6.76 x 10^9 were negative for appendicitis. It seems that the difference between our study results and Khan et al.'s study is attributable to the small sample size of their study and different age groups as they enrolled pediatric patients (25). In a study by Wu et al. to determine the diagnostic value of PCT, CRP, and Alvarado score in appendicitis, the ROC curve (for appendicitis diagnosis) was 0.74 for Alvarado score, 0.69 for PCT, and 0.61 for CRP. In general, the Alvarado score had the greatest potential to the distinction among the three studied tests (26). Another study showed that PCT and D-dimer had a low diagnostic value (26% and 31%, respectively), and positive predictive value of PCT for abscess and/or perforation of the appendix was higher than CRP (73% versus 48%) (27). A study by Motie et al. in Iran showed that PCT at a cutoff point of 0.11 ng/dL had a sensitivity and specificity of 41% and 69%, respectively with positive and negative predictive values of 0.45 and 0.04, respectively (28).

Our study showed that the sensitivity and specificity of WBC in detecting acute appendicitis at the cutoff point of 10 x 10^9 were 79.1% and 86.0%, respectively. Also, the sensitivity and specificity of % PMN in detecting acute appendicitis at a cut point of 65% were 90.7% and 90.7%, respectively. Similar to or results, Anandalwar et al. in a retrospective study in the United States showed that the WBC at a cutoff point of >9 x 10^9 and % PMN at a cutoff point of 65% along with sonography examination, significantly improved the predictive value of sonography in the diagnosis of suspicious appendicitis in children (29). In another study by Afshari et al. the sensitivity of CRP and WBC was 92.77% and 85.54%, respectively. They also showed that the AUC for PCT was 0.421 and the sensitivity and specificity of the PCT test at the best cutoff point was 55.42% and 29.41% respectively, and its overall accuracy was 51%. Hence, PCT had the least diagnostic accuracy among other studied tests (30). In another study on the diagnostic value of different biomarkers in the diagnosis of acute appendicitis in children, it was shown that PCT had a high sensitivity of 95.65% and specificity of 100% in diagnosing acute appendicitis (31).

Our study had some limitations. One limitation was the small sample size and the lack of examination of patients for other factors affecting PCT and WBC levels such as concurrent bacterial infections.

In conclusion, we have shown that PCT is a sensitive test for acute appendicitis but has not sufficient specificity for confirmation of doubtable cases. According to the results of our study and other studies, it is recommended to conduct similar studies with larger sample sizes and increased follow-up periods.

AUTHOR INFORMATION

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Genetic diversity of norovirus among children under 5 years in the South-South region of Nigeria

Favour Osazuwa, Rachel Okojie, Frederick O. Akinbo, William Johnson and Hailey Grobler

ABSTRACT

Background: Norovirus is recognised to be a major cause of gastroenteritis among children worldwide.
Aim: The aim of this study was to determine the genetic diversity of norovirus among children less than 5 years in the South–South region, Nigeria.
Materials and methods: A total of 505 participants, consisting of 405 children with diarrhoea and 100 apparently healthy children, who served as controls, were included in this study. Stool specimens were collected from all participants and analysed for norovirus using conventional reverse transcriptase-PCR. Partial sequencing of the capsid region was performed to genotype the strains.
Results: Norovirus was detected in 45 (11.1%) of children with diarrhoea, norovirus was not detected among control subjects. Genogroup II (GII) norovirus was detected in 38/45 (84.4%) patients, while genogroup I (GI) norovirus was identified in 7/38 (15.6%) patients. Genotype diversity was large, as demonstrated by the nine identified genotypes (2 GI and 7 GII). GII.4 was the most predominant genotype, 24 (63.0%) of all GII norovirus positive samples. Two norovirus GII.4 variants, New Orleans 2009 and Sydney 2012 were identified in this study. A putative novel GII.4 recombinant was also detected.
Conclusion: This study has provided data on norovirus gastroenteritis burden, prevalent norovirus genogroups and genotypes responsible for cases of norovirus infection among children in the South-South region of Nigeria. Provision of appropriate interventions for monitoring and management of norovirus infection are advocated.
Keywords: Norovirus, genotypes, sequencing, children.

INTRODUCTION

Norovirus is a leading cause of sporadic cases and outbreaks of acute gastroenteritis across all age groups in both developing and developed countries (1). Globally, these viruses are known to cause an estimated 1.1 million hospitalisations and up to 218,000 deaths among children less than 5 years annually (2).

Norovirus is a genus in the family *Caliciviridae* and is an icosahedral virus ~38 nm large with an ~7.5 kb single-stranded, positive-sense RNA genome that encodes three large open reading frames (ORF 1, ORF 2 and ORF 3). ORF1 encodes the replicate polyprotein, while ORF2 and ORF3 encode the major and minor capsid proteins, respectively (3). Norovirus exhibits high genetic diversity and can be divided into ten genogroups (4).

Norovirus can be shed in faeces or vomitus from both symptomatic and asymptomatic infected individuals, with an average duration of 10 to 28 days (5). The introduction of rotavirus vaccines throughout the world may have opened a path for norovirus to take over as the leading viral etiologic agent of diarrhoea in children (6). However, while norovirus vaccines are still currently in development, the burden of norovirus disease in many developing nations is yet to be established**.** As a pre-requisite for the introduction of vaccines for norovirus, it is pertinent that the spectrum of the prevailing genotypes of the virus in any environment be determined (7). Therefore, an update on the behaviour and predominant genotype in a given population is needed for the development of effective vaccines. In most of Nigeria, routine norovirus surveillance is not available. There are no data on the genetic diversity of norovirus among children in the South-South region of Nigeria. Also, there are no data on the prevalence of norovirus in symptomatic and asymptomatic individuals in Nigeria. Against this background, this study was conducted to determine the prevalence and genetic diversity of norovirus among children under 5 years with diarrhoea in South–South, Nigeria.

MATERIALS AND METHODS

Study area

This study was carried out at five secondary health institutions (Central Hospital, Warri, Primary Health Care Centre, Pessu/Ugbuwangwe, Central Hospital, Benin City, Stella Obasanjo Hospital, Benin City and Federal Medical Centre, Yenagoa) in Delta, Edo and Bayelsa States of the South-South region of Nigeria. All hospitals are located in the Niger-Delta region of Nigeria, Southern, Nigeria. Warri is the commercial capital of Delta State, Nigeria, with a population of over 311,970 while Yenagoa is the capital city of Bayelsa State with an estimated population of 266,008, and Benin City is the capital of Edo State with an estimated population of 1,495, 800 People (8).

Study population

This study was conducted between March 2018 and February 2019. A total of 505 participants, consisting of 405 children with diarrhoea and 100 asymptomatic apparently healthy age and sex matched children, who served as controls, were recruited for this study. Children with at least 3 episodes of diarrhoea with an onset of 1 to 7 days, whose parents or guardians consented for their ward/children to participate, were included in this study. The exclusion criteria included the refusal of the parents or guardians of wards to give consent, those with other diseases and children on antiviral therapy. A structured questionnaire was administered to collect data on biodata, socio-demographics and clinical symptoms from parents/caregivers of each subject before specimen collection.vThe protocol for this study was approved by the Ethics and Research Committees of the Ministry of Health of Delta, Bayelsa and Edo States (reference numbers CHW/VOL14/130, FMCY/REC/ECC/2017/OC/046 and 732/T/89 respectively).

Specimen collection

Stool specimens were collected into clean universal containers. Supernatant obtained from stool suspension of 50% in 1 ml sterile phosphate buffered saline were stored at -20°C for RT-PCR analysis of norovirus.

RNA extraction

RNA extraction was carried out using AccuPrep® Viral RNA Extraction Kit (Bioneer, Daejon South Korea). In the presence of chaotropic salt, RNA was bound to glass fibers fixed in a column. Proteins and other contaminants were removed through washing steps and the RNA was isolated and eluted in the final elution step (elution volume: 100 μl). Stepwise protocols, as recommended by the manufacturer's instructions, were followed.

Reverse transcriptase –PCR (RT-PCR)
cDNA synthesis

A 20 μl reverse transcription reaction of 1.0μg of extracted RNA (quantitated using nanodrop spectrophotometer) was carried out on 0.2 ml tubes of Accupower RT Premix (Bioneer Corporation, South Korea). The RT/PCR Pre Mix consists of Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV Reverse Transcriptase), an RNA dependent DNA polymerase, and a thermostable DNA polymerase in a lyophilized mix of dNTPs, reaction buffer, RNase inhibitor, a tracking dye, and a stabilizer. The RT reaction was composed of the following steps to generate homogeneous cDNA: incubation at 25°C for 30 sec for primer annealing; heating up to 48°C for 4 min for extension and finally incubation at 55°C for denaturation of the secondary structure of the RNA, followed by heat inactivation at 95°C for 5 min.

Polymerase Chain Reaction

Detection of norovirus was carried out using a previously described method by Kroneman *et al.* (9). Primers used targeted the 5' end of ORF-2, amplifying genogroup-specific sequences of the N-terminal and shell (N/S) region of the VP1 gene. The cDNA generated was then amplified by PCR in a 45 μL reaction mixture containing 0.25 μL each of 10 mMdATP, 10 mMdCTP, 10 mMdGTP, 10 mMdTTP; 10 μL 5X Green Go Taq Buffer; 0.25 μL Taq Polymerase; 30.75 μL RNase free water; 5 μL cDNA template, as described in a previous study (9). For GI noroviruses typing, specific primers G1SKR (C A A C C C A R C C A T T R T A C A) and G1FFN (GGAGATCGCAATCTCCTGCCC) were used while for genotyping GII noroviruses, specific primers GIIFBN (T G G G A G G G C G A T C G C A A T C T) and GIISKR (CCRCCNGCATRHCCRTTRTACAT), for genotyping GIV, specific primers NIFG4F [ATGTACAAGTGGATGCGRTTC and NIFG4P [AGCACTTGGGAGGGGGATCG) respectively, were used in an RT-PCR analysis. To address the issue of false---negative results and to confirm norovirus detection, standard positive controls for norovirus GI, GII and GIV were used under the same conditions as actual clinical samples. 40 cycles of PCR (10 min initial denaturation at 95℃, 30-sec annealing at 48℃, 5 min for an extension at 72℃) and a final extension cycle at 72℃ for 5 min was performed using Master cycler nexus, Eppendorf, Germany. The PCR products were loaded onto 2% agarose gel with 0.5 μg/ml ethidium bromide and electrophoresed in Tris acetic EDTA (TAE) buffer at 100 V for 1 h. The products were visualized on UV illuminator and photographed using a Polaroid camera (10).

Norovirus sequencing

The amplicons from the partial gene regions of the viral capsid genes were purified using QIAquickPCR purification kit (Qiagen Inc., Valencia, CA) Nucleotide sequencing was done using Big Dye ® Terminator v3.1 Cycle sequencing kit (Applied Biosystems, Carlsbad, CA) on 3130 DNA genetic analyser (Applied Biosystems, Carlsbad, CA). Sequences were edited using sequencher® Version 5.4.6 DNA sequence analysis software (Gene codes Corporation, Ann Arbor, MI, USA).

Norovirus genotypes were determined by comparison of corresponding sequences of norovirus strains using the online norovirus genotyping tool version 1.0. available at www.rivm.nl/mpf/norovirus/typing tool.

Phylogenetic analysis

For confirmation of genotyping, nucleotide sequences obtained were aligned with reference sequences using MUSCLE (11). A phylogenetic tree was constructed using the maximum likelihood method based on the Kimura 2-parameter model as implemented in Molecular Evolutionary Genetic Analysis (MEGA) software version 6.0. Bootstrap analysis was used to assess the reliability of grouping using 1,000 pseudo replicates. The distance scale represents the number of nucleotide substitution per position.

Genbank accession numbers

The nucleotide sequences obtained in this study were deposited into the National Centre for Biotechnology Information (Genbank: http://www.ncbi.nlm.nih.gov/) under the accession number MN263857-MN263863 for GI Noroviruses and MN27134-MN271383 for GII noroviruses.

Statistical analysis

The data obtained were analyzed using Chi-square (X^2) test for frequency data and odd ratio analysis for potential risk factors. The statistical software used for all analyses was SPSS v. 16 (IBM Computer Manufacturing Company, NY, USA). Statistical significance was set at the p0.05 level.

RESULTS

Norovirus was detected in 45 (11.1%) of 405 patients with gastroenteritis in this study. Norovirus was not detected among control subjects. In addition, norovirus prevalence among children in the five participatory hospitals was significantly different (P=0.0032) (Table 1). Only norovirus Genogroups I and II were recovered in this study, norovirus Genogroup IV were not detected (Table 2).

Based on capsid gene sequences recovered from the 45 norovirus positive samples, the majority of the GI noroviruses detected in this study belonged to the norovirus GI.3 genotype, 5 (71.4%), while genotype G1.5 were found in 2 patients (Table 3). Seven genotypes of GII noroviruses were detected, genotype GII.4, GII.12, GII.17, GII.6, GII.7, GII.14 and GII.2 were observed in 24, 3, 3, 2, 2, 2 and 2 patients in this study, respectively. Genotyping analysis with the norovirus genotyping tool demonstrated that 12 (50.0%) of the norovirus GII.4 recovered in this study had genetic similarity to the New Orleans 2009 variant, while 10 (41.7%) isolates showed genetic homology to the Sydney 2012 strains. Three genetic clusters of the Sydney 2012 variant were found to circulate among study participants, with varying sequence identity (range 74%-100%). Two GII.4 sequences were unassigned. The two sequences that were unassigned using the genotyping tool were assigned following phylogenetic analysis. One GII.4 isolate (MN271364) did not cluster with either the New Orleans 2009 or the Sydney_2012 GII.4 norovirus reference strains despite been identified to be GII.4, but had phylogenetic similarity with a norovirus strain isolated in 1993 in Bristol (X76716), which itself is a recombinant strain that is neither the New Orleans nor the Sydney 2012 strain. This strain was, however, deemed to be a putative novel recombinant.

Table 1. Norovirus infection among children with diarrhoea in the South-South region of Nigeria.

Location	No. tested by RT-PCR	No. infected (%)	P
Central Hospital, Warri	83	13 (15.7%)	0.0032
Primary Health Centre, Delta State	68	15 (22.1%)	
Federal Medical Centre, Yenagoa	80	4 (5.0%)	
Central Hospital, Benin	99	3 (3.0%)	
Stella Obasanjo Children/Women Hospital	75	10 (13.3%)	

Table 2. Occurrence of Genogroups of norovirus in study participants.

Norovirus Genogroup	Frequency (%)	
	Diarrhoea patients	Control Subjects
Patients		
G I	7 (15.6%)	0
G II	38 (84.4%)	0
G IV	0	0

Table 3. Genotypes of norovirus Genogroup GI and GII.

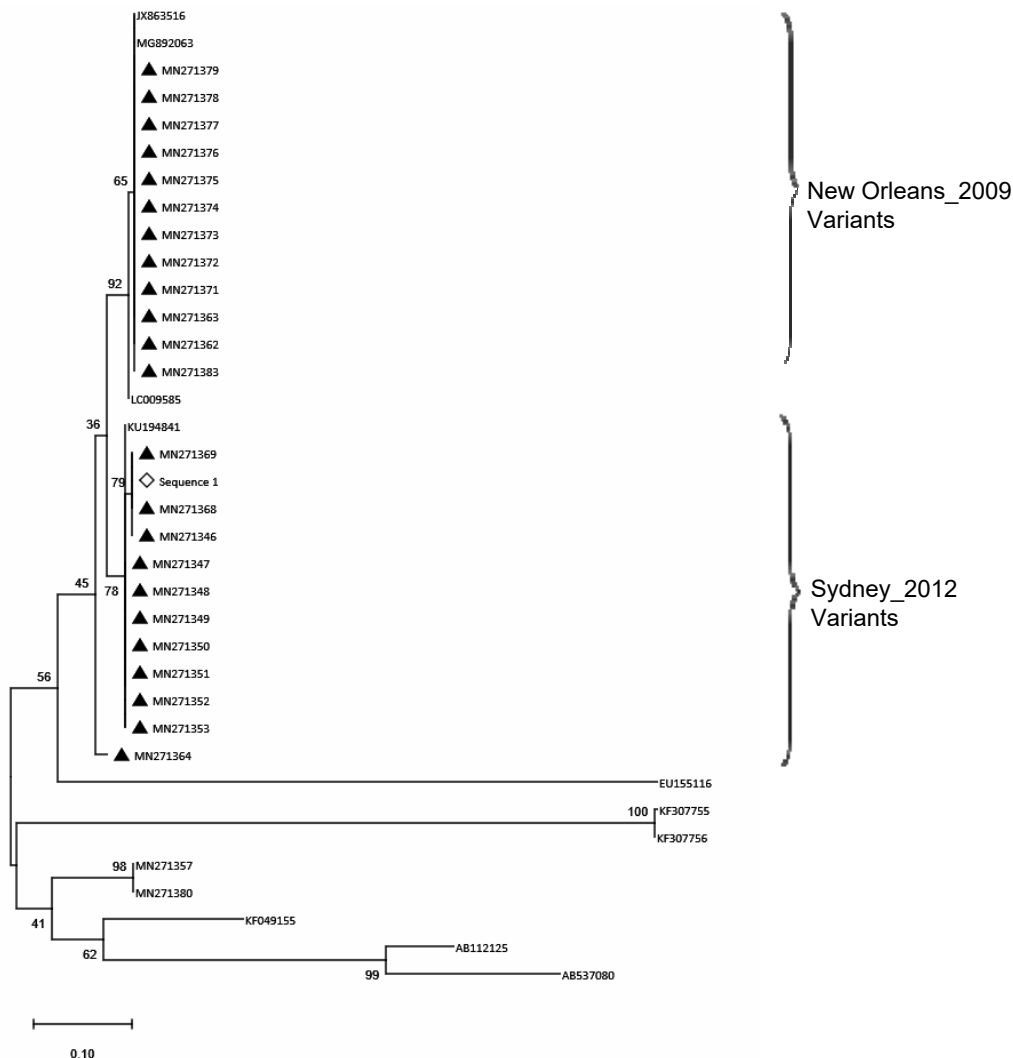| Genotypes		Frequency
G I	GI.3	5 (71.4%)
	GI.5	2 (28.6%)
GII	GII.2	2 (5.3$)
	GII.4	24 (63.0%)
	GII.6	2 (5.3%)
	GII.7	2 (5.5%)
	GII.12	3 (7.9%)
	GII.14	2 (5.3%)
	GII.17	3 (7.9%)

Figure 1. Phylogenetic tree of norovirus GII.4 identified in this study. The evolutionary history was inferred by using Maximum Likelihood method and Kimura 2-Parameter model. The tree with the highest log likelihood is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale with the branch lengths measured in the number of substitution per site. There were a total of 1487 positions in the final dataset. Sequences in this study are represented with filled triangles, reference sequences are not marked. Accession Number KF307755, KF3077756, MN271357, MN271380 were used as outgroup. MN271364 had low bootstrap values, as such classified to be a putative novel recombinant.

DISCUSSION

Noroviruses are important human pathogens known to cause epidemic outbreaks of severe gastroenteritis in communities, military barracks, cruise ships, hospitals and assisted living communities (12). Noroviruses are known to constantly generate genetic and antigenic variants that allow them to evade the host immune system and persist in human populations (13). A prevalence of 11.1% of norovirus infection among children with diarrhoea under 5 years of age was observed in this study, norovirus was not detected among the control subjects. This observed prevalence is higher than the 3.6 % in Edo State and 6.7 % in North East Nigeria (14,15), but less than 37.3% observed among children in Lagos (16). This report supports the hypothesis that the prevalence of norovirus within Nigeria is not homogenous across communities and health centres. Hence, community and hospital-based surveillance is needed to provide an estimate of norovirus burden in Nigeria. The difference in our finding and that of other authors might be due to geographical location, immune status of the host and season of sampling.

Only norovirus belonging to the GI and GII genogroups were identified in this study, this is consistent with previous reports from the South-Western region of Nigeria (17). Norovirus genogroup II was the most commonly recovered norovirus in our study, this finding is similar to previous reports in Lagos, Nigeria (16), Cameroun (18) and South Africa (19). Higher prevalence of norovirus genogroup II has been reported in other African countries, particularly Djibouti, Ghana, Botswana and Burkina Faso (20-23). This study further highlights the predominant role of the norovirus genogroup II in cases of norovirus-induced gastroenteritis among children.

In this study, GII.4 norovirus strains were the most commonly detected genotypes. It has been well established that GII. 4 noroviruses are responsible for the majority of outbreaks worldwide (24). The finding of this study is in agreement with other previous studies where GII.4 noroviruses was the most prevalent genotype among children in different parts of the World; Ife, Nigeria (17), Vhembe district, South Africa (25), in Hongkong (26), and in rural Bangladesh (27).

Phylogenetic analysis revealed two GII.4 sequence variants, two Sydney 2012 clusters and one cluster for the New Orleans 2009 strain and a putative recombinant GII.4 virus. It is established that new variants of GII.4 norovirus emerge every 1-2 years (28), due to genetic recombination and point mutations resulting in the generation of new genetic clusters, recombinants/genotypes allowing increasing genetic fitness and continuous spread in populations by evading host immune responses (29). GII.4 possesses the largest number of intra-genotypic variants and recombinants (30). It is also possible for two or more variants to co-circulate at the same time in a geographical location (31). Notable GII.4 variants causing majority of pandemic diseases are the Sydney 2012, New Orleans 2009, Farmington Hills 2002, US95/96 1995, Hunter 2004 and the Den Haag 2009 (32).

Norovirus GIV was not detected in this present study. This finding shows that GIV norovirus is an uncommon norovirus genotype in our study area. Norovirus was not detected among the control subjects recruited for this study, the reason for this finding is unclear. The shedding of norovirus in stool by asymptomatic individuals has been well documented in some previous studies by other researchers (33-35). It is important to note that in those studies, symptomatic individuals had a significantly higher prevalence of norovirus when compared to asymptomatic subjects. Also, norovirus shedding was significantly longer in symptomatic individuals than in asymptomatic individuals. The findings of our study imply that routine screening of norovirus among healthy individuals might be of no significance in determining norovirus burden in our study area.

The major limitation of this study was that P typing was not done for noroviruses identified among study participants. Dual typing of both capsid and polymerase regions are now recommended for proper classification of noroviruses, this is in accordance with the updated classification scheme provided by the international norovirus working group in 2019 (4). P typing could not be done in this present study because it was not included in the protocol of this study during proposal design. Conceptualisation and design of this study was concluded in 2017. Also, the sample size for asymptomatic controls included in this study was small, this was occasioned by lack of funds. Further studies incorporating a larger number of healthy subjects are needed to properly determine the prevalence of norovirus among asymptomatic individuals.

In conclusion, prevalence of RT-PCR confirmed norovirus infection among children with gastroenteritis in our locality was 11.1%. Norovirus was not detected among control subjects. Norovirus genogroups I and II were the noroviruses recovered in this study. GII.4 norovirus was the most prevalent norovirus genotype detected, other genotypes of norovirus were also encountered. The GII.4 variants observed in our study is believed to belong to the New Orleans 2009 and Sydney 2012 strains, a putative novel GII.4 recombinant was recovered in our study. Regular molecular epidemiologic monitoring for drug and vaccine designs and planning of eradication program is advocated.

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Vitamin B$_{12}$ and folate levels in the cord blood of the newborn at Middlemore Hospital

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ABSTRACT

Objectives and methods: Vitamin B$_{12}$ (Vit B$_{12}$) and folate are essential micronutrients for early neural development in the fetus. Two hundred and forty-four healthy newborn participants were enrolled over ten months into a study that tested cord blood levels of Vit B$_{12}$, folate and haemoglobin (Hb). Informed consent was obtained from the mothers of each newborn and samples tested for Vit B$_{12}$, folate and Hb at Middlemore Hospital, Auckland, New Zealand.

Results: Newborn Vit B$_{12}$ levels ranged from 36.9 and 1476.0 pmol/L with a mean of 361.3 pmol/L providing a 95% confidence interval reference range of 112.0 - 871.9 pmol/L ($p = 0.59$). Cord blood folate ranged from 1.36 and 45.4 nmol/L with a mean of 35.2 nmol/L and a 95% CI range of 15.5 - 45.0 nmol/L ($p = 0.72$). The Hb results ranged from 84 to 271 g/L with a mean of 161.3 g/L and a 95% confidence interval reference range of 121.5 - 209.5 g/L ($p = 0.17$). There was a positive correlation between the cord blood Hb and Vit B$_{12}$, $R^2 = 0.81\%$ ($p = 0.08$), whereas little correlation existed between elevated cord folate and Hb levels ($p = 0.51$). A statistically significant difference was identified in folate cord blood levels in New Zealand Maori and other Polynesians in comparison to the other ethnic groups.

Conclusions: This study revealed that Vit B$_{12}$ and folate levels at birth are similar to levels seen in adults at this hospital. Results from the various ethnic groups included in the study showed some variation in Vit B$_{12}$, folate and Hb levels. Also, an elevation in cord blood Hb was associated with elevated Vit B$_{12}$ levels.

Keywords: Vitamin B$_{12}$, folate, micronutrients, newborn.

INTRODUCTION

Vitamin B$_{12}$ (Vit B$_{12}$) and folate are micronutrients vital for cellular DNA synthesis, cellular division and cell metabolism. They are particularly important for the developing brain and other neural tissues in the fetus and the newborn. Maternal deficiency of Vit B$_{12}$ and folate during pregnancy have been implicated in fetal developmental complications, such as neural tube defects and megaloblastic anaemia. To prevent these disorders, pregnant women are recommended to augment their dietary intake with supplementary Vit B$_{12}$ and folate (1). In New Zealand, recommendations for dietary supplementation in pregnancy include: 0.8mg folate daily for at least four weeks before and twelve weeks after conception, and the consumption of folate-rich foods (2). For Vit B$_{12,}$ the recommended daily intake is 2.6 mcg to 2.8 mcg per day. Neural tube defect and other conditions linked to maternal deficiencies of Vit B$_{12}$ and folate during pregnancy were reported in 2001 occurring at a rate of 3.4 per 10,000 births in New Zealand (2).

Vit B$_{12}$ and folate deficiency-related issues in the newborn are likely to go unconfirmed due to the lack of blood testing at the time of birth. In addition, there is insufficient information published about levels of Vit B$_{12}$ and folate in otherwise healthy neonates (3). The purpose of this study was to investigate Vit B$_{12}$, folate and Hb levels in neonates born at Middlemore Hospital, and to establish if a difference exists in these micronutrients in the ethnic groups in the area.

Middlemore Hospital, as part of the Counties Manukau District Health Board, is located in South Auckland in New Zealand (4). The region is home to many New Zealand Europeans, New Zealand Maori, other Polynesian and Asian populations. Significant health inequities exist among these communities (5,6).

MATERIALS AND METHODS

This was a prospective observational study of a cohort of neonates from the Middlemore Hospital birthing unit. Babies from uncomplicated deliveries who did not require extendedneonatal care, and babies with no known health issues were included. Identical twins were counted as a single participant. The study was approved by the Massey University Human Ethics Committee: Human Ethics Southern A Committee (SOA 16/43) on 12th October 2016. Further approval was granted by the Counties Manukau Health Research Committee (Research Study 25).

Enrolment and sampling

Recruitment of participants occurred between October 2016 to August 2017. There were 60 neonatal participants from four main ethnic subgroups (New Zealand European, Maori, Pacific Island and Asian). Enrolment was sought following the arrival of a new neo-natal cord blood sample in the Blood Bank. The principal investigator then approached potential participant mothers in the Maternity ward. Maternal informed consent was obtained and information on the neonate's ethnicity (Maori, Pacific Island, Asian, European or other), as well as information regarding any dietary supplementation used during pregnancy.

Cord blood analysis

Ethylene diamine tetra-acetic acid (EDTA) anticoagulated cord blood samples were stored at 4°C, and the separated plasma was used for Vit B$_{12}$ and folate testing in the clinical biochemistry laboratory. Both assays employed an electrochemiluminescent immunoassay utilising the Roche Cobas analyser (Roche Diagnostics, Germany) (7). For the folate assay there was an upper limit cut-off of 45.4 nmol/L, as values above this are not able to be accurately measured (8). A paired cord blood sample was tested for a complete blood count using the Sysmex XN10 haematology analyser (Sysmex, Japan).

Statistical analysis

Results were analysed using 'Minitab' and 'R' statistical software, and reference intervals were calculated to provide 95% prediction intervals based on the fitted distribution, two-sided for Vit B$_{12}$, and one-sided for folate due to the limitations on the measurement of the upper limit (9-11).

Multiple distributions were tested to identify the best-fit distribution using QQ-plots, simulations and Kolmogorov-Smirnov tests. Parameters were estimated using quantile matching estimation (0.25 and 0.75). The best-fit distribution was defined as the distribution that best captured the 0.05 to 0.95 quantiles of the samples. The Kruskal-Wallis Rank Sum test was used to compare the results from the ethnic groups.

RESULTS

Of the mothers approached, 95% provided consent for their child to be tested. A total of 244 participant samples (including three sets of identical twins, counted as a single sample) were tested producing 243 completed sample analyses. The gestation period for the neonates ranged from 32 to 40 weeks.

The range for the cord blood Vit B_{12} was 36.9 to 1476.0 pmol/L (mean 361.3 pmol/L). For folate, the range was 1.36 to 45.4 nmol/L (mean 35.2 nmol/L). The Hb ranged from 84 to 271 g/L (mean of 161.3 g/L).

A regular log normal distribution was found to be the best fit for Vit B_{12} (p: 0.59) and a modified normal distribution (to account for the fixed upper limit) was the best fit for folate (p: 0.72). Figures 1 and 2 show QQ-plots of measurements against the best-fitted distribution. Based on fitted distributions the prediction intervals for Vit B_{12} was 112.0 to 871.9 pmol/L and folate 15.5 nmol/L or greater. The 95% prediction interval for neonatal Hb results was shown to be 121.5 to 209.5 g/L. Table 2 provides a summary overview with matched reference intervals.

A total of 21.8% of the participants identified as Maori, 31.3% as Pacific Islander, 32.1% as Asian and 46.5% as European; – 17 ethnic combinations were identified. Table 1 shows Vit B_{12} and folate levels for the ethnic groupings.

There was no significant difference for Vit B_{12} (p: 0.23) between the single or combined ethnic groups; however, a difference for folate levels was identified (p: 0.02). For New Zealand Maori and Pacific Islanders, or those with a combination of the two ethnicities, the mean folate was lower at 29.1 nmol/L for Maori and 31.8 nmol/L for Pacific Islanders as compared to 35.9 nmol/L for European and 37.2 nmol/L for Asian (p: 0.001) groups respectively. No statistically significant difference was noted among non-Maori or non-Pacific Islander ethnicities (p: 0.42) or between those identifying solely as Maori or Pacific Islander (p: 0.32) (Figure 3).

Table 1. Overview of the distribution of ethnicity, Vitamin B_{12} and folate.
IQR = interquartile range from first (Q1) to third (Q3) quartile.

Overview of the distribution of Vitamin B_{12} and Folate levels											
		Vitamin B-$_{12}$					Folate				
Ethnicity	N	Mean (median)	Min	IQR (Q1;Q3)		Max	Mean (median)	Min	IQR (Q1;Q3)		Max
Total	243	361.3 (295.1)	36.9	220.0	;443.8	1476.0	35.16 (38.01)	1.36	27.34	;45.40	45.40
Maori	11	401.1 (360.1)	153.0	293.8	;423.2	851.4	29.08 (31.82)	11.84	22.08	;38.70	41.22
Pacific Islander	39	341.2 (278.1)	88.7	209.8	;470.8	1027.0	31.84 (33.48)	13.15	21.88	;43.39	45.40
Asian	58	397.5 (356.5)	36.9	237.4	;488.7	1476.0	37.22 (42.92)	1.36	30.56	;45.40	45.40
European/ Other	64	339.8 (266.7)	121.6	189.8	;394.5	1476.0	35.92 (37.31)	15.70	28.71	;45.40	45.40
Pacific Islander & Maori	7	407.7 (272.7)	231.2	257.9	;522.7	788.5	28.83 (27.33)	12.80	23.56	;34.58	45.40
European & Maori	23	316.6 (318.1)	103.1	218.0	;397.9	592.6	37.20 (38.73)	19.68	31.24	;45.26	45.40
2 other ethnicities	25	360.5 (289.0)	125.6	216.3	;449.6	929.9	36.07 (36.74)	15.00	30.04	;45.40	45.40
Three or more ethnicities	16	383.8 (283.3)	180.2	235.4	;583.1	732.7	35.41 (37.92)	19.10	29.47	;42.12	45.40

Table 2. Reference intervals for neonates from the study and the reference intervals for the adult population of Counties Manukau.

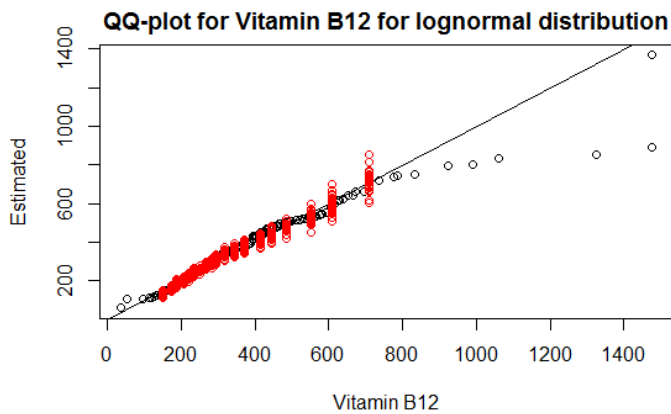| Newborn 95% reference intervals		
Reference interval	Lower limit	Upper limit
Vitamin B_{12} (pmol/L) CMDHB adult rf range: 130 - 800	112.0	871.9
Folate (nmol/L) CMDHB adult rf range: 7.0 - 45.0	≥15.5	n/a
Hb (g/L) CMDHB cord blood rf range: 124 - 192	121.5	209.5

Figure 1. QQ-plot of Vitamin B12 against its fitted distribution. Black spots are the measured values and the red are values of the 0.05 to 0.95 quantiles by 0.05 intervals of 25 simulated samples from the fitted distribution.
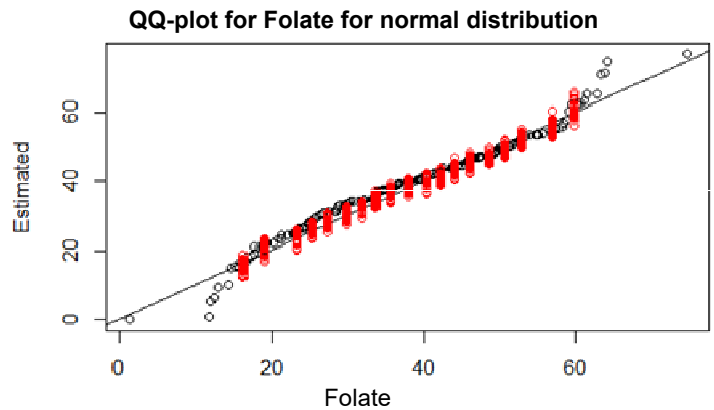

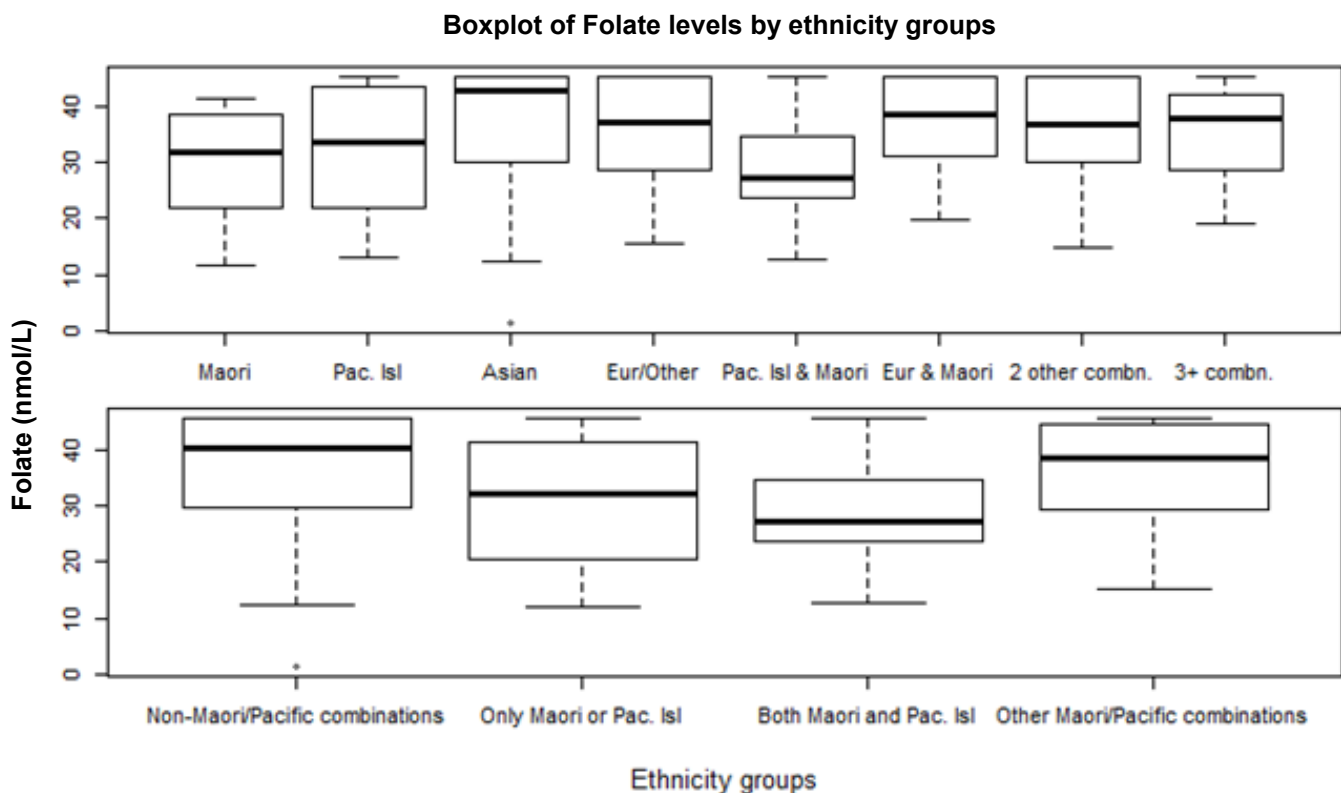**Figure 2**. QQ-plot of folate against its fitted distribution. Black spots are the measured values (mirrored over its median) while the red are values of the 0.05 to 0.95 quantiles by 0.05 intervals of 25 simulated sampled from the fitted distribution.

Figure 3. Boxplot of the levels of folate across the various ethnicity groups, defined by the HiSO sole/combined output for ethnicity and by Maori and Pacific Islander versus non-Maori-non-Pacific Islander groups.

DISCUSSION

A lack of published information regarding Vit B_{12} and folate levels in the newborn and the diverse ethnic mix of the South Auckland region provided an opportunity to undertake this research. This prospective observational single center study included 244 healthy neonates delivered without complication between 32 to 40 weeks of gestation. Collected maternal ethnicity data was diverse, as was the intake of dietary supplements, that included Elevit, iodine, iron, folate and other vitamins. Some mothers were on diabetes medication and /or aspirin. Most mothers were informed of the importance of iron, Vit B_{12} and folate in pregnancy and most were taking dietary supplements.

The mean Vit B_{12} at 361.3 pmol/L provided a reference interval of 112.0 - 871.9 pmol/L. This is both higher and wider than the adult reference range. An elevated serum Vit B_{12} correlated with an elevated cord blood Hb. The mean cord blood folate at 35.2 nmol/L provided a reference interval of ≥ 15.5 - 45.0 nmol/L compared to the folate adult reference range of 7.0 - 45.0 nmol/L. No significant statistical association was identified between folate and the cord blood Hb. The mean cord blood Hb at 161.3 g/L (121.5 - 209.5 g/L) compares closely to the existing cord blood reference range (124 - 192 g/L).

Two low Vit B_{12} results were identified in the study and were referred to the co-investigator consultant haematologist (HB) and to neonatologists in the neo-natal intensive care unit. Both babies were South Asians (Indians). Case 1: reduced Vit B_{12} at 40.74 pmol/L, folate normal at 45.40 nmol/L and Hb 133 g/L. Case 2: Vit B_{12} at 36.90 pmol/L, folate low at 1.36 nmol/L and cord blood Hb 136 g/L. As Vit B_{12} and folate testing are rarely undertaken on cord blood samples, the physicians were unsure of what these results meant. Both babies were discharged from the Maternity Unit with a plan to follow-up with haematology testing and to monitor neural development.

There were 17 ethnic infant combinations enrolled in the study. Statistical differences between single and combined ethnicities for Vit B_{12}, folate and Hb were evaluated using the Kruskal-Wallis Rank Sum test. No significant difference was noted for Vit B_{12} or Hb levels, However, there was a difference noted in folate levels between Maori and Pacific Islanders versus the New Zealand European and Asian population groups.

Previous studies have suggested that newborn babies of mothers from a lower socio-economic background have lower folate levels as compared to those with a mother of higher socio-economic status. In a study of Brazilian neonates, mothers from a low socioeconomic background tended to present with lower folate levels when compared to women from a higher socioeconomic group (12). In our study, only maternal ethnicity was established. Overall only minor differences were noted in the results from the different ethnic groups studied.

CONCLUSIONS

Our study demonstrated that differences in Vit B_{12} and folate levels among the newborn of the ethnically diverse population of the South Auckland region were small. The study also showed that reference values for Vit B_{12} and folate for healthy term infants were similar to those for the adult population of the region. There were few (4 low Vit B_{12}, 1 low folate) deficient cord bloods identified. A positive association between elevated cord blood Hb and Vit B_{12} values was found. The high rate of dietary supplementation (99%) by the mothers demonstrates good public awareness of the importance of micronutrient supplementation in pregnancy.

The work undertaken in this study focused on a small group of neonates from Middlemore Hospital in the Counties Manukau region of New Zealand. Future follow-up studies might in the future include a larger sample of the New Zealand population.

ACKNOWLEDGMENTS

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Refeeding syndrome: a case study

Natasha Dutt and Samarina MA Musaad

ABSTRACT

This case study describes a case of hyponatremia and hypochloraemia caused by refeeding syndrome. Refeeding syndrome is a constellation of symptoms and signs caused by metabolic disturbances that occur as the body switches from the catabolic state associated with malnutrition to the anabolic fed state after re-introduction of caloric intake. It is characterised by deranged phosphate, potassium, magnesium, and water balance within the body. Awareness of refeeding syndrome, its risk factors and biochemical findings are important to enable timely management.

Key words: refeeding syndrome, metabolic disturbance, starvation, hypophosphatemia, hypokalaemia, hypochloraemia.

INTRODUCTION

Refeeding syndrome is a poorly recognised and understood condition with no universally accepted definition (1). It occurs after unbalanced re-introduction of caloric intake following prolonged starvation and has distinct metabolic disturbances (2). These disturbances are best understood in context of catabolic processes associated with starvation, and anabolic processes associated with refeeding (3). The metabolic consequences are characterised by deranged phosphate, potassium, magnesium, and fluid balance within the body (3). Early reports of refeeding syndrome were prisoners of war, hunger strikers, alcoholics, and individuals with anorexia nervosa reported in more recent times (4).

Hypophosphatemia is the hallmark of refeeding syndrome, but it can also occur in other conditions such as kidney disorders, diabetic ketoacidosis, chronic diarrhoea, vitamin D deficiency and severe malnutrition (5,6). Severe hypophosphatemia (0.2-0.36 mmol/L) can cause respiratory failure (4). Additionally, hypomagnesemia, hypokalaemia, hyponatraemia and thiamine deficiency can also occur with refeeding syndrome (7). However, it is not clear whether thiamine deficiency is a result of refeeding or a pre-existing deficiency due to starvation (3). Chloride levels often parallel sodium levels but hypochloraemia has not been highlighted as a critical finding in refeeding syndrome even though it is lost from the gastrointestinal tract by vomiting and/or diarrhoea that may be associated with prolonged states of malnutrition (8). Hypochloraemic metabolic alkalosis is associated with hypokalaemia in cases of prolonged vomiting due to the loss of hydrogen ions (9). In the early stages, refeeding syndrome may be asymptomatic, before it progresses to nonspecific symptoms such as generalised muscle weakness. If not managed in a timely manner symptoms and signs worsen and include seizures, heart failure, tachycardia or arrhythmias, high blood pressure, coma and potentially death (10).

The prevalence of refeeding syndrome varies based on criteria used for diagnosis. In a prospective observational study of 178 individuals admitted acutely to the department of internal medicine in a teaching hospital, 97 (54%) of individuals were found to be at risk of refeeding syndrome based on the NICE criteria (1). The mean age was 66.8 years (+/- 17.4 years). Fourteen (14%) of these individuals developed refeeding syndrome based on severe hypophosphatemia (< 0.60 mmol/L); this constituted 8% of the total 178 admitted. Incidence amongst intensive care patients is around 34% (11). Refeeding hypophosphatemia was found in 10.4% of malnourished patients in a Veterans Administration Hospital (12), and in a prospective cohort study in a teaching hospital, on 243 adults

started on artificial nutrition for the first time, the incidence of refeeding syndrome was found to be 2% (1). The smaller incidence in the latter population may have been partly due to in-hospital planned management of artificial nutrition.

Endocrine and biochemical processes

The direct source of energy for physiological functions in the body is glucose (10). It is principally regulated by the interplay between insulin and glucagon (5). Insulin is an anabolic hormone that increases in proportion to glucose promoting its entry into cells and its storage as glycogen, mainly in the liver (10). When glucose levels drop as occurs during fasting state, glucagon rises to maintain blood glucose levels by breaking down glycogen, the storage form of glucose, in the liver, a process called glycogenolysis (7).

The main source of glucose is carbohydrates with smaller but important contributions from fats and proteins. Starvation deprives the body of glucose, amongst other nutrients, and starts a cascade of hormonal changes aimed at maintaining fuel for bodily functions, including decreasing levels of insulin and increasing levels of glucagon and the stress hormones; growth hormone and cortisol (13). Ultimately, as glycogen stores are depleted, glucose is sourced from gluconeogenesis promoted by rising growth hormone and decreasing insulin levels (5). Gluconeogenesis produces glucose from molecules such as amino acids, lactate, pyruvate, and glycerol in the liver (14). Glycerol is a product of increased lipolysis, whereby the body resorts to breakdown of stored fats promoted by growth hormone and insulin (13). Lipolysis also produces free fatty acids and an increase in ketone body production; free fatty acids are used by muscle and other tissues, while the brain uses ketone bodies as a source of energy (2).

Refeeding syndrome occurs when food and energy are re-introduced with a shift from lipolysis, protein break-down and gluconeogenesis associated with catabolism to the anabolic fed state (5). This abrupt shift in metabolism induces a series of profound biochemical changes within the body (3). The introduction of glucose stimulates insulin secretion which promotes the intracellular shift of glucose and supports anabolic processes: glycogenesis, lipogenesis and protein synthesis, while reducing the rate of glycogenolysis and gluconeogenesis (7). Additionally, insulin secretion stimulates phosphate, potassium and magnesium to move to the intracellular compartments (7). An increase in demand for electrolytes and water and their inter-compartmental shifts cause serum levels to fall, leading to critical complications (15). Figure 1 summarises metabolic processes involved in starvation and refeeding (2,15).
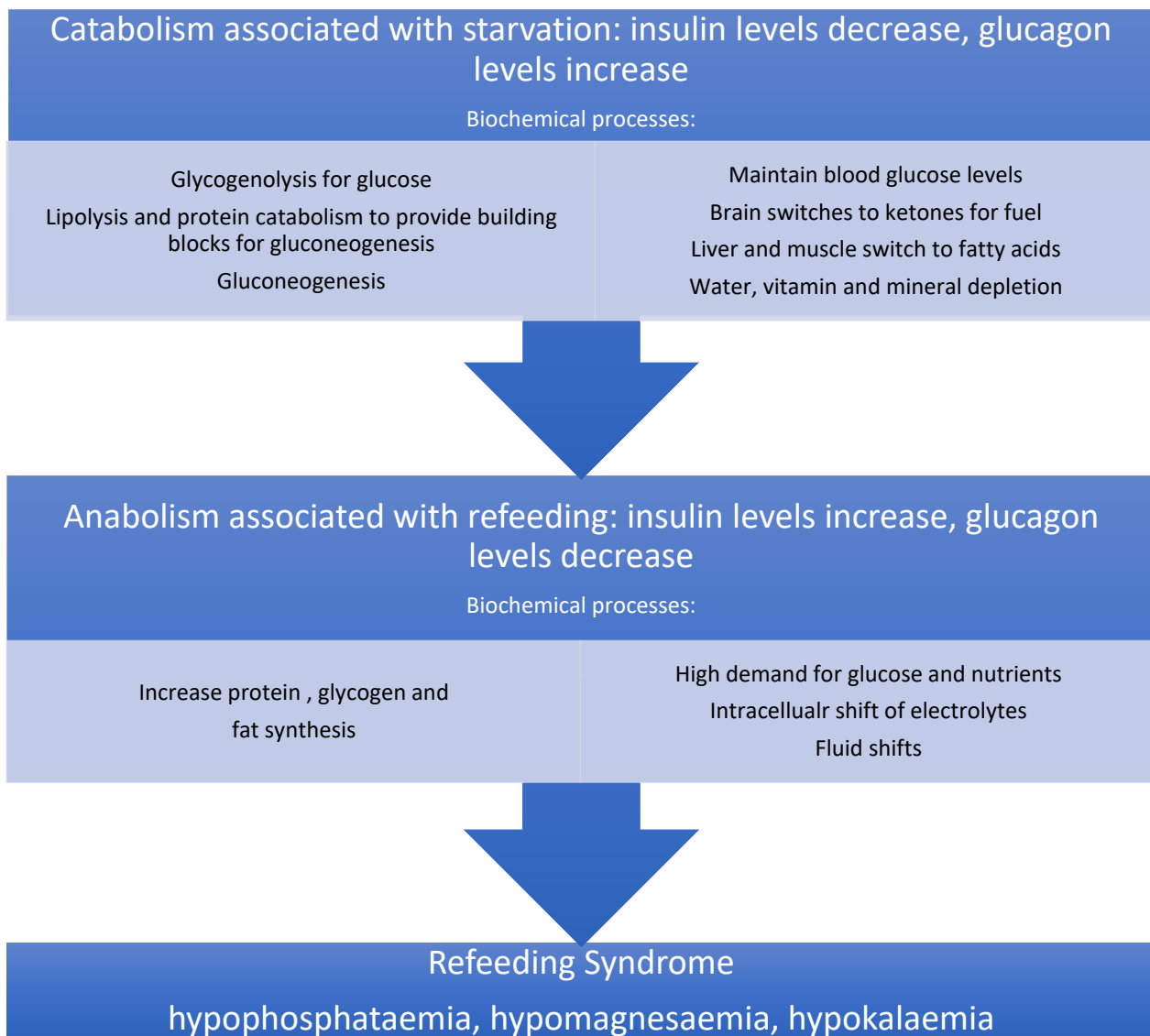
Figure 1. Metabolic processes involved in starvation and refeeding. Adapted from Crook *et al*. (2) and Stanga *et al*. (15).

We present a case of hypophosphatemia and hypochloraemia with a history of prolonged low energy intake. Hyponatraemia and hypokalaemia are not uncommon in community laboratory practice but this degree of hypochloraemia had not been seen previously by the authors.

CASE STUDY

Mrs X was a 73-year-old visitor to New Zealand with a history of duodenal ulcer treated conservatively four months prior to her arrival in New Zealand. She had on-going nausea and reduced food intake resulting in a loss of 12 kg over two months. Clinical notes did not indicate whether she had vomiting, diarrhoea, oedema, or other relevant symptoms. She had a recent duodenostomy (a connection between two parts of the duodenum to bypass an obstructed segment) for duodenal stenosis performed in the private sector in New Zealand. She had a background history of reflux oesophagitis, ischaemic heart disease and congestive heart failure, hyperlipidaemia and hypertension, but no known history of diabetes mellitus.

Mrs X was prescribed antihypertensive medication ramipril (an angiotensin converting enzyme inhibitor) (16); pantoprazole (a proton pump inhibitor) for reflux oesophagitis (17) and multivitamins with thiamine hydrochloride. Table 1 summarises blood levels three days after surgery (Day 1).

On Day 1, the low sodium was signalled to the on-call scientist as "critically low" since laboratory protocol states that less than 125 mmol/L is considered a critical result. The result was therefore communicated to the requestor who admitted Mrs X acutely to hospital where she was diagnosed with refeeding syndrome.

DISCUSSION

Risk factors for developing refeeding syndrome include severeweight loss, alcoholism, chronic diseases causing undernutrition, for example cancer postoperative patients, chronic low energy intake and long-term use of antacids and anorexia nervosa (10). Table 2 summarises risk factors based on the National Institute for Health and Care Excellence (NICE) guidelines.

Table 1. Timeline of renal function tests for Mrs X.

	RR	Lab A (out-patient)	Lab B (in-patient)	Lab B (in-patient)	Lab B (in-patient)
		Day 1	Day 1	Day 2	Day 5
Sodium mmol/L	135-145	124	123	129	132
Potassium mmol/L	3.5-5.2	3.1	2.5	3.5	3.7
Chloride mmol/L	95-100	70	71	79	91
Phosphate mmol/L	0.70-1.50	0.61	0.37	1.00	0.93
Magnesium mmol/L	0.70-1.00	1.12	1.1	0.96	0.85
Bicarbonate mmol/L	22-31		>40		

RR: Reference range; Lab A: Laboratory A; Lab B: Laboratory B. Note: source from clinical notes.

Table 2. NICE criteria for determining patients at risk of developing refeeding syndrome. Kraaijenbrink BV, *et al*. (1).

One or more of the following:
Body Mass Index < 16 kg/m^2
Unintentional weight loss > 15% within the last 3-6 months
Little or no nutritional intake for more than 10 days
Low levels of phosphate, potassium, or magnesium prior to feeding
Or two or more of the following:
Body Mass Index < 18.5 kg/m^2
Unintentional weight loss > 10% within the last 3-6 months
Little or no nutritional intake for more than 5 days
History of alcohol abuse or drugs including insulin, chemotherapy, antacids, or diuretics

Mrs X had a 12 kg weight-loss and recent surgery. The clinical notes did not indicate her previous weight nor her current body mass index (BMI) but a loss of 12 kg over eight weeks can be significant (depending on her original weight) and can put her at high risk for refeeding syndrome. She was starved for weeks prior to surgery and was not eating well post-surgery which would have resulted in decreased skeletal, cardiac and respiratory muscle mass as well as overall strength (8). With malnourishment, patients become total body phosphate depleted, and while it was the sodium that was critically low, she did have a moderately low potassium of 3.1 mmol/L which dropped further to 2.5 mmol/L and a low phosphate of 0.61 mmol/L which also dropped further to 0.37 mmol/L. Insulin production increased with re-introduction of food, and the intracellular shift of phosphate would have aggravated her already low phosphate levels.

Clinical details do not indicate whether Mrs X was vomiting pre- or post-surgery. However, reflux oesophagitis can predispose to vomiting because of oesophageal mucosal injury due to the reflux of gastric acid and pepsin (18). Pepsin is an enzyme that helps with breakdown of proteins in the stomach. The past history of duodenal ulcer may predispose to the narrowing of the duodenum which led to inserting a stent to fix the obstruction. Also, of note are side effects of pantoprazole, which include diarrhoea, stomach pain, nausea and vomiting (17).

In general, chloride ion is not prioritised when assessing electrolyte disturbances, partly because potassium and sodium can be critical at identifying and diagnosing metabolic complications. Hypochloraemia and hyperchloremia can be caused by vomiting and dehydration, respectively (9).

Hypochloraemia in our patient may be explained by severe malnutrition and it is possible that she did in fact suffer from vomiting that was not mentioned in the brief clinical notes (8). Metabolic alkalosis, as seen in Mrs X (bicarbonate of more than 40 mmol/L), is commonly seen with hypochloraemia in those who vomit where there is loss of chloride ions due to upper gastrointestinal tract loss of hydrochloric acid and stomach secretions (18). Vomiting also leads to loss of hydrogen ions causing a relative increased delivery of bicarbonate ions to the kidneys with an increased excretion of potassium resulting in a net metabolic alkalosis, hypokalaemia with the hypochloraemia (19).

The magnesium result is incongruent with the usual biochemical findings in refeeding syndrome. Mrs X had slightly high magnesium result which could be due to the multivitamins she was prescribed. Furthermore, it is a possibility that she consumed over the counter magnesium salts for heart-burn (20). Magnesium is a co-factor for many metabolic processes and plays an important role in protein synthesis and normal muscle, cardiac and nerve functions (7).

OUTCOME AND CONCLUSION
Mrs X was given oral potassium and intravenous phosphate replacement. She was discharged after three days during which her metabolic abnormalities resolved. Mrs X was asked to continue with her multivitamins as prescribed prior to her admission. We have discussed a case of refeeding syndrome in a high-risk female with a long-standing history of inadequate caloric intake and significant weight loss. She had some of the classic biochemical features of refeeding syndrome and notable hypochloraemia.

Take home messages

- Chloride may not be considered as critical of an analyte as sodium or potassium, but it can alert the scientist to serious metabolic derangement and may play an important role in conditions, such as refeeding syndrome.
- Further research needs to be conducted to better understand hypochloraemia and if there is any correlation with refeeding syndrome.
- Hypophosphatemia is the hallmark of refeeding syndrome.
- Critical alert limits are important tools in support of timely management.
- It is imperative to recognise patients who are prone to refeeding syndrome and understand the physiologic mechanisms involved in starvation and refeeding.
- It is important to design and follow clear critical result processes aimed to facilitate timely management.

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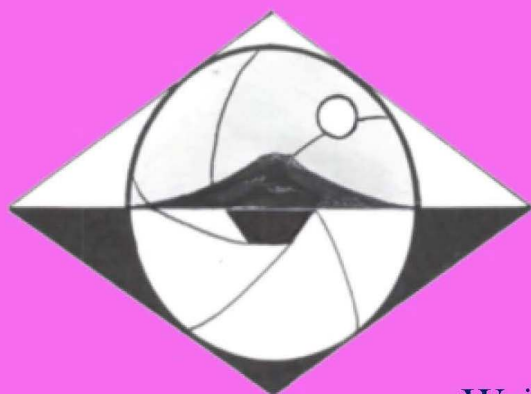
20 / 20
Visions of the Future
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Medical laboratory science; the distortion of nomenclature across the globe

Dear Editor,

Medical laboratory science is the professional practice that involves analysis of human or animal tissues, body fluids, excretions, production of biologicals, designs and fabrication of equipment for the purpose of medical laboratory diagnosis, treatment and research. The Medical Laboratory Science Council of Nigeria (MLSCN) Act 11 2003 (1) describes the profession as encompassing medical microbiology (bacteriology, parasitology, virology, mycology), clinical chemistry (chemical pathology), haematology & blood transfusion science, histopathology, forensic science, molecular biology, laboratory management, or any other related subject as may be approved by the appropriate authorities like the regulatory body (2) MLSCN as an instance.

Medical laboratory testing is an integral part of quality health care which provides physicians, nurses, and other healthcare providers with objective information that aids in patient care. This specialty helps in identifying risk for developing diseases, early disease detection, planning disease management strategies, selecting safe and effective treatments options, monitoring treatment responses, pinpointing threats to patients safety and public health concerns, protecting the blood supply and transplants recipients from harmful pathogens, adverse transfusion reactions and assays for drugs of abuse to support clinical care as well as ensure public safety.

A professional practicing medical laboratory science is called a medical laboratory scientist. Apart from the fact that it has the longest name as a professional, unlike nurse, Doctor, pharmacist, engineer, etc., the nomenclature is distorted across the globe. The distorted nomenclature reads biomedical scientist in the UK, to clinical scientist, clinical laboratory scientist, medical laboratory technologist, medical scientist, medical technologist, medical laboratory scientist in the USA and other countries of the world.

The internationally recognized body of the profession is the International Federation of Biomedical Laboratory Science (IFBLS). However, different nomenclature exists in various countries depending on the adopted nomenclature. This therefore connotes that the profession is an evolving one which should be given a global attention both in quality and nomenclature.

This is important considering the professional role in healthcare. Akuyam (3) and Obeta *et al*. (2) do not leave any stone unturned with regards to the roles of the medical laboratory scientist in healthcare, which includes provision of accurate laboratory results in a timely manner aiding 60-70% of all decisions regarding patients treatment; providing accurate information to those with the responsibility for treating patients; guides physicians and nurses in choosing the correct laboratory tests and proper sample collection methods; performs equipment validation in the laboratories; plays a role in critical diseases surveillance; plays a critical role in clinical and public health decisions; as well as prognosis; contributes immensely to quality assurance of a health facility and generally provide possible laboratory research for possible cost improvement or reduction in patients' diagnostic aspect of healthcare proceedings based on technology used and type of diagnosis used in the medical laboratory.

According to Ejilemele and Ojule (4) without reliable medical laboratory support; patients are less likely to receive the best possible care; resistance to essential drugs will continue to spread; the source of disease may not be identified correctly; the spread of communicable disease will not be checked reliably; and the valuable financial and human resources may be diverted to ineffective treatment and control.

It is therefore imperative to challenge the professionals across the globe as individuals or organizations like the IFBLS to come up with a strategy to harmonise the nomenclature, curriculum and practice towards an enviable status like their counterparts the doctors, nurses, pharmacists and other health professionals.

We sincerely acknowledge the efforts of the IFBLS in uniting the professionals across the globe and improving quality of medical laboratory science practice. However, it is time to reach a consensus on the nomenclature and title of medical laboratory science practitioners across the globe to ensure uniformity of purpose, practice and professionalism.

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BARRIE EDWARDS AND ROD KENNEDY SCHOLARSHIP REPORT
Rebecca O'Toole, Wellington SCL

I was a very proud recipient of the Barry Edwards and Rod Kennedy Scholarship which enabled me to attend an international conference to present my scientific work. The conference I chose was the European Congress on Thrombosis and Haemostasis (ECTH), held in Glasgow, United-Kingdom (UK), from 2-4 October 2019.

This was a relatively small conference (600 people) with the same calibre of speakers as other international events. The advantage of a smaller event is that you can easily mingle with the other presenters, providing a platform to build networks and set up connections and collaborations.

A wonderful introduction to the conference was the first plenary lecture by Gordon Lowe, a semi-retired Professor of Vascular Medicine at the University of Glasgow. He took us on an impressive journey through time to showcase the history of Scottish medicine. Among these was the first documented clinical trial in history by James Lind (1753) which evaluated the effectiveness of citrus fruit against scurvy. Other landmark achievements attributed to the Scottish are the introduction of aseptic procedures in the operating theatre, inventions such as the polygraph test, ultrasound and full-body MRI, as well as discoveries such as insulin and penicillin. Scotland also pioneered the world's first neurosurgery and kidney transplant. The list went on and on. For what Lowe described as a "small, cold and wet" country, it has a proud medical history with developments in science and medicine which have had global impact.

Emicizumab (Hemlibra®), a new treatment in patients with Haemophilia A (PwHA), took centre stage at the conference. This bispecific antibody bridges activated factor IXa and Factor X to replace the function of missing activated Factor VIII (FVIII) and restore haemostasis. Emicizumab offers numerous advantages over FVIII replacement therapy, the current standard of care. It has a longer half-life (30 days compared to 8-12 hours) so doses are less frequent, and it can be administered sub-cutaneous rather than intravenously, which is problematic in young patients. A further treatment challenge is the development of inhibitory antibodies against replacement FVIII, blocking its action and causing inferior outcomes for PwHA. This novel drug has a different mode of action and does not cause or increase inhibitors and furthermore remains active in their presence. Therefor FDA approval of Emicizumab represents a significant milestone in reducing the burden of care for this disease.

Michael Callaghan presented a study on the long-term efficacy and safety of emicizumab in PwHA across the four phase III studies: HAVEN 1-4. It was concluded that emicizumab maintained low bleed rates and favourable safety and tolerability long term in a broad population of PwHA regardless of age, FVIII inhibitor status, or dosing regimen. A reduction in the annualized treated bleed rate was reported, as well as an increase in the proportion of participants with no treated joint or spontaneous bleeds.

The surgical experiences from HAVEN 1-4 trials were presented by Guy Young. In total, there were 214 minor and 19 major surgeries performed. For minor surgeries, such as dental, it was found that emicizumab alone provides good haemostatic coverage. In the majority of these, prophylactic coagulation factor was not used, and of these, >90% did not result in a treated post-operative bleed. On the contrary, prophylactic coagulation factor was used routinely in the majority of major surgeries (*n = 16*) and only one resulted in a treated post-operative bleed. There was no post-operative bleeding in the three major surgeries managed without prophylactic coagulation factor. Importantly, no procedure resulted in death, thrombosis, FVIII inhibitor development, or unexpected bleeds.

Individual case stories were also presented by Michael Callaghan, Gerry Dolan and Guy Young which highlighted the burden of Haemophilia A, e.g. daily FVIII intravenous infusions from an early age (e.g. one year old) and the difficulties in administering and adhering to the regime; frequent uncontrolled and spontaneous bleeds, debilitating joint damage and pain, as well as the limitations imposed on normal activity caused by worry about bleeds. For these families, routine accidents like cuts and scrapes can be distressing and life-threatening. Emicizumab was life-changing in each of the cases presented, cutting treatment times from multiple time-consuming infusions every week to a single injection given sub-cutaneously once-a-week / fortnight / month. Inhibitors did not develop, and it dramatically reduced the risk of bleeds. In each case presented, the drug significantly improved the quality of life of patients and their families.

For laboratories of course, this revolutionary drug represents a different challenge as emicizumab interferes with the one-stage FVIII assay routinely used for monitoring PwHA. A chromogenic factor VIII assay using bovine factor IX and X substrates is required since emicizumab is specific for human FIX and FX. This was a topic raised in questions again and again and formed the subject of many of the posters on display. The answer was always the same, a suitable validated chromogenic FVIII assay must be accessible routinely for PwHA on emicizumab and laboratories need to get ready.

The highlight by far for me was the "Meet the expert" sessions which took place early in the morning before the lectures began. This formula for these sessions was informal – there were no powerpoints involved, letting the questions from the attendees lead the conversation. With laboratory and clinical representative from all over the world, it was informative to hear insights while sharing our triumphs and of course our tribulations.

This gave me the chance to meet Dr Steve Kitchen, a name which requires no introduction on the Coagulation circuit. He is a clinical scientist at the Sheffield Haemostasis and Thrombosis Centre, has a long-standing interest in standardisation and is very active drawing up guidelines within the International Society on Thrombosis and Haemostasis (ISTH), as well as other international bodies in the field.

One common problem faced by coagulation laboratories worldwide seems to be the aging / retiring of experts in the field. In some organisations this is coupled with financial pressure to make the service cheaper by employing less staff at a lower grade, hence lower level of expertise. Rather than this short-sighted approach, we talked a lot about ways in which you can save money in the overall healthcare budget by investing in the laboratory. One such example Steve Kitchen gave was how his team convinced the Renal Unit and Haematology medical units in his hospital to fund the development of ADAMTS13 Activity assay on-site (staff time, reagents etc). Sending the test away took between 2-8 days and had it been on-site, 20-30% of patients who were currently going straight onto plasmapheresis would not have needed it as they did not have Thrombotic Thrombocytopenia Purpura. In the UK plasmapheresis costs £5-10K per day so this resulted in substantial savings per patient in Sheffield where they see an average of four suspected cases per year. Essentially, laboratories may need to do some convincing that quality coagulation testing is not in the end expensive, it may require investment but will save money elsewhere in the service e.g. patient bedtime.

We also talked about ways to improve efficiencies using HIL checks, IT system alerts (rules) and protocols which ultimately save time and reagents. Phone time and subsequent lack of standardisation of clinical decision-making when it comes to

reflex testing was pinpointed as one source of inefficiency in laboratories. The approach taken to resolve this problem at Sheffield is a protocol for an isolated prolonged activated partial thromboplastin time (APTT) which involves automatic progression from screening tests to diagnostic assays without the need to contact a clinician until the ultimate diagnosis is reached. What is of interest is that this protocol is so robust that mixing studies becomes effectively redundant, while meeting the fundamental goal of detecting severe factor deficiencies and acquired haemophilias in a timely and cost-effective manner.

Another hot topic was demand management of coagulation screen request. Several studies have demonstrated the very low efficacy of routine pre-operative coagulation screens for the prediction of bleeding risk. In line with the British Committee for Standards in Haematology (BCSH) recommendations, Sheffield successfully replaced the systematic prescription of routine coagulation tests with a structured questionnaire (a truncated version of the ISTH bleeding assessment tool, designed for picking up von Willebrands Disease (VWD) and platelet disorders). Kitchen says that they have found in three years, they have picked up more patients with VWD using this protocol and they now do 5000 less coagulation screens per year (out of a total 100K). For the pre-operative units alone, this is a reduction of 50%.

These sentiments were not shared at another presentation I attended on the same topic in which it was claimed that neither a standardised questionnaire nor routine coagulation tests have acceptable performances to detect haemostatic abnormalities before surgery. A multicentre study by Ajzenberg *et al*. was presented in which 1,405 patients were included across seven French academic hospitals over a three-year period. Each patient completed a questionnaire and had prothrombin time (PT), APTT, platelet count, von Willebrands Factor (vWF) activity and antigen, factors VIII, IX and XI, platelet function

assay (PFA) performed and when required, Factors II, V, X and VII, and haemostasis consultation. The questionnaire used was that of the French Society of Anaesthesiology and Intensive Care. The diagnostic performance of the two strategies was then compared: standardized questionnaire versus routine coagulation tests. Of the 1,405 patients, 16 (1.14%) had haemostatic abnormalities potentially associated with bleeding risk. Sensitivity of the questionnaire was 50% and the specificity 87%, which is in adequate to detect bleeding abnormalities. Of note, however, was that the sensitivity of the bleeding questionnaire among women alone was 80% with VWD being the most common diagnosis (7/10 subjects). Sensitivity and specificity of the routine coagulation tests strategy were 75% and 51% respectively. Concordance between the two strategies was very low. Since the most frequent abnormality was VWD, future insights aim to look at the performance of the combined questionnaire and vWF activity to see if it is better than the questionnaire alone.

It is impossible to summarise all the presentations and topics of the ECTH conference into one report, but these were the ones that stood out for me. Overall, this experience I feel is the kind of opportunity that comes along once in lifetime and has brought me more value than I could have imagined. Hearing perspectives from scientists in other laboratories was thought-provoking and illuminating. In a small country like New Zealand such interactions have a catalytic effect on our continuous professional development. Secretly, however, I did take pride in the fact that many of the initiatives being discussed are well established in our laboratories here and we seemed to be very much on par and in many cases, ahead of the game. I would like to thank the NZIMLS for facilitating this trip for me. I am extremely grateful and feel truly privileged to have received it. If anyone has any questions about the conference please feel free to contact me at rebecca.otoole@wellington.co.nz.

2020 Annual Scientific Meeting Social Programme

Icebreaker / Opening of the Exhibition

The welcome function will give you the opportunity to catch up with friends and colleagues whilst enjoying drinks and nibbles. It is also a special time for our exhibitors to host this function around their exhibits.

Date	Tuesday 11th August 2020
Time	6.30pm to 8.30pm
Venue	Banquet Rooms 1 and 2
Cost	Complimentary to full delegates, sponsors and exhibitors $40 per additional ticket

Poster Session

Date	Wednesday 12th August 2020
Time	5.00pm to 6.00pm
Venue	Horizon Lounge
Cost	Complimentary to full delegates, Wednesday delegates, sponsors and exhibitors $30 per additional ticket
Authors	Poster authors will be available to speak about their work

Networking Function

Date	Thursday 13th August 2020
Time	5.00pm – 8.30pm
Venue	Banquet Rooms 1 and 2 and Horizon Lounge
Theme	The kinkiest glasses
Dress	Conference attire
Cost	Included within the cost of registration for full delegates only $65 per additional ticket

THE Pacific WAY

PACIFIC PATHOLOGY TRAINING CENTRE

Centre based training courses

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.

Blood transfusion November 2019

The above course commenced on the 4th Nov and concluded on the 29th Nov 2019. Seven students attended:

Boote Maritino	Kiribati
Rozeleen Chand	Fiji
Natasha Philip	PNG
Dr Ari Jayanti Pereira Tilman	Timor Leste
Estefania Pacheco	Timor Leste
Sina Reti	Samoa
Ray Bulemaru	Vanuatu
Lecturer: Susan Evans	

Pacific Pathology Training Centre training courses for 2020

- Laboratory health & safety; and quality management systems
  9 March – 3 April 2020 (4 weeks)

- Biochemistry
  20 April – 15 May 2020 (4 weeks)

- Effective laboratory management
  25 May – 19 June 2020 (4 weeks)

- Haematology and blood cell morphology
  29 June – 7 August 2020 (6 weeks)

- Microbiology
  24 August – 18 September 2020 (4 weeks)

- Blood transfusion science
  19 October – 13 November 2020 (4 weeks)

For further information contact:

Navin Karan,
Programme Manager
Pacific Pathology Training Centre
Wellington
New Zealand
P: +64 4 389 6294   E: navink@pptc.org.nz

Can you help?

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

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

Please contact:

Phil Wakem
Chief Executive Officer
Pacific Pathology Training Centre
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 April 2020 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 19 June 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

APRIL 2020
JOURNAL QUESTIONNAIRE

1. Limitations of prostate specific antigen testing has resulted in what?

2. What are the possible mechanisms of antimicrobial resistance cases?

3. Which types of glycopeptide resistance genes have been described in *enterococci* and which are the most important?

4. How can vancomycin resistance genes be transmitted and potentially cause what?

5. What is the principle of the cyanmethemoglobin photometric method for determining haemoglobin?

6. What are currently the most important diagnostic criteria for appendicitis?

7. Norovirus is a virus with a RNA genome that encodes open reading frames. What do those frames encode?

8. Maternal deficiencies of Vitamin B12 and folate during pregnancy have been implicated in which fetal developmental complications?

9. What are the New Zealand recommendations for dietary supplementation of Vitamin B12 and folate in pregnancy?

10. The metabolic consequences of refeeding syndrome are characterised by what processes in the body?

NOVEMBER 2019
JOURNAL QUESTIONNAIRE
AND ANSWERS

1. What are the current components and their roles of the Ziehl-Neelsen stain?
   Carbol fuchsin as the primary stain, acid-alcohol as the decolourising agent and methylene blue as the counter 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?
   Family of Ca2+ binding proteins and helps to regulate the amount of intracellular calcium. It has been identified in high concentrations in Schwann and Astrocyte cells and can also be detected in amniotic fluid, urine and cord blood.

3. How do carbapenemase-producing *Enterobacterales* confer resistance to carbapenem antibiotics?
   Via the ability to hydrolyse the beta-lactam ring in carbapenem structures.

4. What were the five most common bacteria isolated from students' mobile phones?
   Bacillus species, *Acinetobacter lowfii,* coagulase-negative *staphylococcus*, inactive *Escherichia coli* and *Enterobacter agglomerans*.

5. What conditions could result from a high level of plasma homocysteine, and what could it result in?
   Thrombophilia, vascular endothelial cell injury and toxic effects on the embryo. Could result in pregnancy loss.

6. Hyperhomocysteinemia resulting in endothelial damage has a direct influence on what?
   Atherogenesis, activation of coagulation factor V and VII, increased thrombin and platelet aggregation.

7. Which factors have been identified as important modifiable metabolic syndrome risk factors?
   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.

8. The mechanisms through which smoking reduces HDL-C have been linked to alteration of which lipid transport enzymes?
   By reducing lecithin-cholesterol acyl transferase activity, lowering cholesterol ester transfer protein and hepatic lipase activity.

9. Which common haemoglobin variants can interfere with some HbA1c assay methods?
   Hb S, Hb C, Hb E and Hb D.

10. Which methodologies have the highest detection rate of haemoglobin variants and derivates, and which have the least detection rate?
    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.

BARRIE EDWARDS & ROD KENNEDY SCHOLARSHIP

The Barrie Edwards and Rod Kennedy Scholarship is one of the most significant awards offered by the NZIMLS. The scholarship provides the successful applicant with support to attend an international or national scientific meeting up to a maximum value of $7,500.

Applications for this prestigious scholarship are invited from Fellows, Members and Associate Members of the NZIMLS. Applicants must be a current financial member of the NZIMLS and have been a financial member for at least two concurrent years prior to application. To be eligible applicants must make an oral presentation or present a poster as 1st author at the nominated scientific meeting.

Barrie Edwards   Rod Kennedy

All applications will be considered by a panel consisting of senior medical laboratory scientists (who are ineligible to apply for the scholarships). The applications will be judged on the quality of the presentation and your professional and academic abilities together with your participation in the profession. The panel's decision is final and no correspondence will be entered into either during or after the decision making process. In the event of the presentation being declined by the conference organisers the successful Scholarship applicant must notify the NZIMLS Executive Officer immediately. It is strongly recommended that no financial commitment is made until the notification of the conference abstract outcome is known as the Scholarship award may be required to be returned to the NZIMLS.

Evidence of travel insurance is required following all travel bookings and conference arrangements.

Application is by using the Scholarship Application form and Curriculum Vitae form. All correspondence and applications should be sent to:

NZIMLS Executive Officer
PO Box 505
Rangiora 7440

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

Successful applicants will be required to provide a full written report within three months on return from the conference, which will be published in the New Zealand Journal of Medical Laboratory Science. If not intending to publish elsewhere, successful applicants will be required to submit their study results for consideration by the New Zealand Journal of Medical Laboratory Science within 12 months following the conference.

Acknowledgement of the Barrie Edwards and Rod Kennedy Scholarship in both the presentation and any subsequent publication is a required condition of this award. The NZIMLS Council reserves the right to request the full conference presentation.

	Previous Recipients	**To Attend**
2019	Pippa Dryland LabPLUS, Auckland	CSLI DDC Committee meeting, USA
2018	Jacquie Wright ESR, Upper Hutt	VTEC Meeting, Florence, Italy 2018
2017	Rebecca O'Toole Wellington SCL, Haematology	3rd European Congress on Thrombosis and Haemostasis, Glasgow, United Kingdom 2-4 October 2019
2015	Maxine Reed Aotea Pathology	Oral Presentation at the AACC Conference, Atlanta, Georgia, USA July 2015
2014	Julie Creighton Microbiology, Canterbury Health Laboratories	Poster Presentation at the ICAAC 2014, Washington DC, USA
2013	Mary Stevens Canterbury Health Laboratories	Poster Presentation at the American Society of Microbiology Conference, Denver, Colorado, USA 18-21 May 2013
2012	Holly Perry School of Applied Science, AUT University, Auckland	Oral Presentation at the American Association of Blood Banks Conference, Boston USA 2012
2011	Bernard Chambers Haematology, Middlemore Hospital, Auckland	Poster Presentation at the IBMS Biomedical Science Congress 2011, United Kingdom
2010	Sandy Woods Canterbury Health Laboratories. Christchurch	Oral Presentation at the NZIMLS/AIMS South Pacific Congress 2011, Gold Coast, Australia

NZIMLS Research Grant

A Research Grant of up to $5,000.00 is available to applicants who:

- Have been a member of the New Zealand Institute of Medical Laboratory Science (Inc.) for a minimum period of two years
- Are currently employed in an IANZ accredited laboratory
- Have fully completed the Application Form (below)

Applicants are encouraged to supply a final written report to the NZIMLS Journal Editor (within 12 months of completion of the project) for consideration of publication in the Journal of Medical Laboratory Science.

The decision of the NZIMLS Council on the awarding of Grants is final, and no correspondence will be entered into.

Application for the NZIMLS Research Grant is to be completed in full on the forms available at:
https://www.nzimls.org.nz/research-grant

	Previous Recipients	Project
2019	Casey O'Byrne Wellington SCL, Wairarapa	An investigation of the potential significance of the *Arcobacter* species; *Arcobacter butzleri, Arcobacter cryaerophilus,* and *Arcobacter skirrowii* as emerging enteric pathogens in New Zealand
2018	Jacquie Leaman Canterbury SCL	Prevalence testing for Mycoplasma genitalium and Trichomonas vaginalis on Hologics Panther platform

NZIMLS Journal Prize

Council of the NZIMLS has approved an annual Journal prize to the value of NZ$300 for the best peer-reviewed article published in the Journal during the calendar year. The article can be a review article, original article, case study, research letter, or technical communication. Excluded are Fellowship dissertations.

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

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

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

No formal application is necessary but you must be the 1st author and a current financial member of the NZIMLS to be eligible. All articles accepted and published during the calendar year (April, August and November issues) will be considered. The Editor, Deputy Editor and the President of the NZIMLS, who themselves are ineligible, will judge all eligible articles in December. Their decision will be final and no correspondence will be entered into.

Winners of the 2019 Journal prize are Keyleigh Hancock and Karen McKinley from SCL Invercargill for their article "Very long chain acyl-coenzyme A dehydrogenase deficiency. A case study". *N Z J Med Lab Sci* 2019; 73: 14-18.

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, Australasian 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)

Index to Volume 73, 2019

OBITUARY

AUTHOR INDEX

2020 NZIMLS CALENDAR *Dates may be subject to change*		
DATE	**COUNCIL**	**CONTACT**
7/8 May	Council Meeting, Hamilton	fran@nzimls.org.nz
DATE	**SEMINARS**	**CONTACT**
9 May	North Island Seminar, Jet Park Airport Hotel, Hamilton	melissa.niemandt@waikatodhb.health.nz
22-24 May	NICE Weekend, Wairakei Resort, Taupo	raewyn.cameron@pathlab.co.nz
20 June	Pre-Analytical Seminar, Waipuna Conference Centre, Auckland	ajesh.joseph@waikatodhb.health.nz
6 November	Immunology SIG Seminar, Jet Park Airport Hotel, Hamilton	andrew.soepnel@waikato.health.nz
DATE	**CONFERENCE**	**CONTACT**
11-14 August	Annual Scientific Meeting Waipuna Hotel and Conference Centre, Auckland	fran@nzimls.org.nz
DATE	**MEMBERSHIP INFORMATION**	**CONTACT**
15 June	Material for the August Journal must be with the Editor	rob.siebers@otago.ac.nz
18 June	Nomination forms for election of Officers and Remits to be with the Membership	fran@nzimls.org.nz
8 July	Nominations close for election of officers	fran@nzimls.org.nz
26 July	Ballot papers to be with the membership	fran@nzimls.org.nz
01 August	Annual Reports be with the membership	sharon@nzimls.org.nz
09 August	Ballot papers and proxies to be with the Executive Officer	fran@nzimls.org.nz
15 September	Material for the November Journal must be with the Editor	rob.siebers@otago.ac.nz
DATE	**NZIMLS EXAMINATIONS**	**CONTACT**
07 November	QMLT Examinations	fran@nzimls.org.nz

NZIMLS invites you to attend the

North Island Seminar

to be held at

Jet Park Airport Hotel,
Hamilton

Saturday 9 May 2020

Want to present?
Then we want to hear from you!

Email your interest to Melissa Niemandt:
Melissa.Niemandt@waikatodhb.health.nz

Awesome people wanted!

Registration 8:15am—9:00 am with tea and coffee

Seminar commences at 9:00am with a 5:00pm finish

Followed by drinks and nibbles

Registration available at

www.nzimls.org.nz
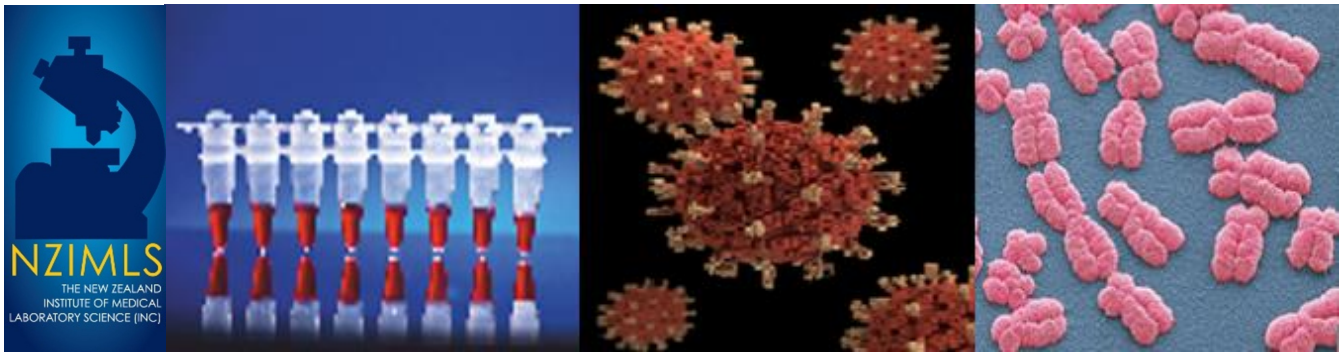
NZIMLS presents the

Molecular Diagnostics SIG Meeting
Friday, 26 June 2020

Christchurch Hospital, Manawa Building,
276 Antigua Street, Christchurch

Presentations (oral and poster) are invited from the following disciplines:
Translational Diagnostic Research, Molecular Genetics,
Biochemical Genetics, Molecular Virology, Molecular Microbiology,
Molecular Haematology, Cytogenetics

Closing date for abstracts: Friday 15 May 2020

For further information contact:
genelab.test@cdhb.health.nz

Online Registration at: www.nzimls.org.nz

NZIMLS Presents the

Microbiology Special Interest Group Seminar

NZIMLS
THE NEW ZEALAND INSTITUTE OF MEDICAL LABORATORY SCIENCE (INC)
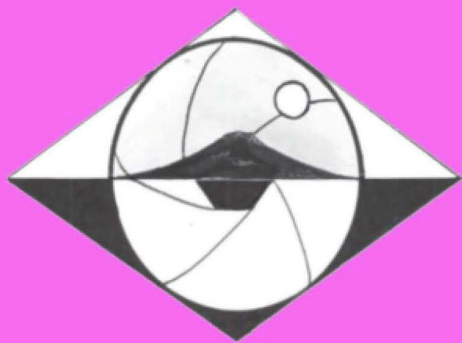
ARA Institute of Canterbury Madras Street Christchurch

Saturday 27 June 2020

Register at: www.nzimls.org.nz

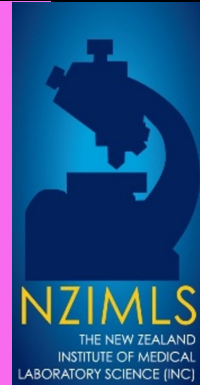Contact for presentations: Jacquie Leaman Jacquie.leaman@sclabs.co.nz

20 / 20

Visions of the Future

NZIMLS Conference
and Annual Scientific Meeting

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

Join us!
11—14 August 2020
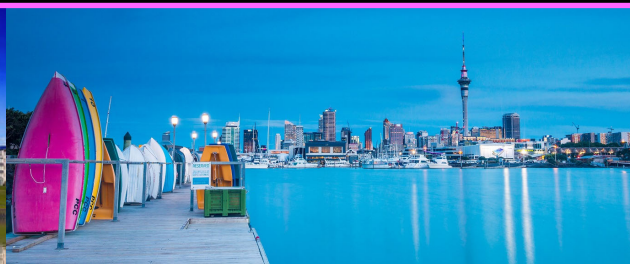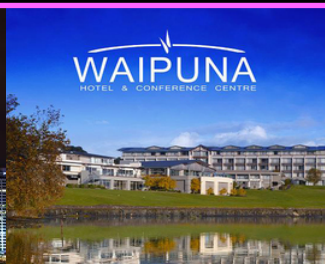# at
Waipuna Hotel and
Conference Centre
Auckland

Conference Convenor:
Tracey Camp
E: tbathgate@adhb.govt.nz

Registration is available at
www.nzimls.org.nz

Delphic V10

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

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

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

Simplified
implementation,
configuration and
support

Modernised
technology stack

Enhanced
lab business
management

Delivering
new functionality
and modules

Intuitive
user experience

Streamlined
lab workflow