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In this issue

Rob Siebers, Editor

The development of resistance to antimicrobial agents among Gram-negative pathogenic organisms has been progressive. Pathogen of particular concern are the extended-spectrum β-lactamase (ESBL)-producing ones. Since the ESBL-producing organisms are posing major threat for clinical therapeutics, it is mandatory to identify the prevalence of such strains in hospitals. In this issue Sobia and colleagues from India investigated the incidence of the beta-lactamase producing Enterobacterial strains *Escherichia coli* and *Klebsiella pneumoniae* over a three year period. They showed that the *bla* gene, bla_{ampC}, declined in the three years while bla_{ESBLs} prevalence showed a slight increase in one year, but in the following year prevalence was was very similar to the initial year. The authors conclude that surveillance of carbapenemase-producing organisms seems to be essential for proper implementation of infection control strategies and also for selection of appropriate antimicrobial therapy.

Haemolysis is a major source of errors in the clinical laboratory. In this issue Lippi and Ippolito from Italy investigated the effects of spurious haemolysis on prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen in 12 patients on warfarin therapy and in 12 healthy subjects not taking any medications. They found that values of all coagulation parameters remained unchanged in haemolyzed aliquots of both healthy subjects and patients on warfarin therapy, with no significant difference compared to the baseline aliquot without haemolysis. The authors conclude that results of PT, APTT and fibrinogen in patient samples containing up to 3.6 g/L of cell-free hemoglobin are reliable.

Medical laboratory workers are at occupational risk of contracting blood borne viral diseases. In this issue Olandeinde and colleagues assessed the frequency and factors associated with uptake of human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) testing services among 110 medical laboratory scientists in Nigeria. The authors found that all scientists reported to have had at least one post qualification HIV screening test. However, only 37.3% and 15.5% of scientists had been screened for HBV and HCV respectively. They conclude that uptake of HBV and HCV testing services was poor and that regular screening for HIV, HBV and HCV is advocated for medical laboratory scientists in Nigeria.

Chromophobe renal cell carcinoma (ChRCC) is a variant of parenchymal renal cell carcinoma, comprising 2-5 % of all renal cell carcinomas and characterised by unique morphological, histochemical, ultrastructural and genetic features In this issue Lallu and colleagues from Wellington report two cases of ChRCC which were confirmed by cytomorphology along with a Hale's colloidal iron stain and immunohistochemical and FISH analysis.

Heparin-induced thrombocytopenia (HIT) is a complication of heparin use due to development of HIT antibody which activates platelets resulting in a prothrombotic state. Early diagnosis and management is essential to prevent critical ischaemia or even death resulting from uncontrolled thrombosis. A rapid, sensitive and specific functional laboratory diagnostic test, however, is not available in most hospitals. In this issue Chan and colleagues from Auckland present a preliminary simple assay based on the drop in platelet count of normal donor platelet-rich plasma in the presence of HIT antibody and heparin. The authors conclude that this proof-of-principle finding suggests this simple test, which can be done in any haematology laboratory with a modern blood cell counter, holds great promise as a simple functional test for laboratory diagnosis of HIT. Further work is required to validate the results.

In this issue in an Editorial Mike Legge from Dunedin presents the up to date status of the Clinical Scientist qualification from the Faculty of Science of the Royal College of Pathologists of Australasia (RCPA) which will be of great interest for medical laboratory scientists in New Zealand contemplating this potential new employment category in the medical laboratory.

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Editorial
The dawn of the clinical scientist

Mike Legge

Members of the NZIMLS will be aware of the discussions, conference presentations and printed articles relating to the concept of a new employment category – the Clinical Scientist. Over the last six or so years there has been active dialogue relating to this development, which moved to a closer reality when the Royal College of Pathologists of Australasia (RCPA) made a majority decision by it's Fellows to support the establishment of the Faculty of Science as part of the RCPA. To establish the Faculty of Science, senior scientists were invited to apply as Founding Fellows and a number of senior scientists particularly in Australia and New Zealand were appointed as Founding Fellows of the Faculty of Science based on their research publications, roles in education and the profession. Overall 299 Founding Fellows were admitted and this route is now closed.

From this cohort and in conjunction with the RCPA staff and Pathologists the Faculty of Science Committee was formed which had the responsibility of establishing rules, pathways to Fellowship, writing discipline curricula, formulating strategy, educational standards etc. This collaboration between the RCPA and the fledgling Faculty of Science has been outstanding with considerable will to see the whole process to completion and develop plans for the future of the Faculty. As a result of the meetings and discussions two main routes to Fellowship are now established, either by a five- year training programme or by assessment of published peer reviewed research. For the five-year programme (which would be the normal route) entry would be at the Bachelors' degree level plus five years laboratory experience. On acceptance, the vocational training period in a specific discipline would commence that would normally be for 2-years when the Part 1 assessment and examination would be taken. Successful completion would gain entry to the 3-year Part 2 vocational training programme with the Part 2 assessment and examination at the end of this period. Successful completion of Part 2 gains Fellow of the Faculty of Science [FFSc(RCPA)]. Entry with an Honors degree or suitable MSc may reduce the pre-requisite pre-training period by 1 to 2 years. Applicants with a suitable PhD would be required to have a minimum of 2-years post-doctoral experience. Accommodation for 'prior learning' against the curriculum will be possible for the Part 1, for appropriately qualified candidates who may only need to complete part of the requirements (and final examinations) if it can be demonstrated equivalence via publications, management or alternative examinations, and would be assessed on a case-by case basis. Scientists with a PhD and 30 or more peer-reviewed publications may apply for Fellowship directly and are again assessed on a case-by-case basis by the Faculty.

Overall the training programme has three requirements: Clinical Laboratory, Research, and Leadership, Innovation and Development. The requirements for each of these are detailed in the discipline curricula. Currently discipline curricula are available from the RCPA web-site (www.rcpa.edu.au) for: Anatomical Pathology (including Cytology), Chemical Pathology, Genetics, Haematology (including Transfusion Science) and Microbiology. Each of these details the requirements, standards, course outlines and training programmes for each discipline. Immunopathology is close to completion and two additional curricula are under development, Forensic Science and Bioinfomatics/Informatics. Besides the educational and experience entry requirements, the training laboratory must be accredited by the RCPA as appropriate for training. In the first instance this would be a laboratory, which already has Pathology Registrars training for their FRCPA. Each student will require two supervisors, a FRCPA and an FFSc (RCPA); although in special circumstances one of the supervisors may not be a Fellow of the RCPA, but requires approval. Application forms for consideration to enter the programme are available on the RCPA web-site.

Issues still to be resolved, particularly in New Zealand, relate to future registration, funding of training positions, and negotiations relating to training time in diagnostic laboratories, provision of suitable research facilities, and future career opportunities. Currently there are 18 FFSc(RCPA) in New Zealand spread across the major disciplines in the main centres. Preliminary indications are that there is interest from at least another 30 scientists who may be eligible to apply for the training programme via higher degree/the prior learning route. All application information including the Trainee Handbook is available on www.rcpa.edu.au.

The concept of the Clinical Scientist is well established in the UK and is considered to bridge the gap between medically qualified Pathologists and scientists with advanced training. It follows therefore that Clinical Scientists (FFSc) are senior, experienced scientists who are capable of working in conjunction with medical specialists in the scientific disciplines of pathology and are an integral part of the health care system. It would be anticipated that, although the traditional disciplines in pathology will attract some trainees, the newer areas such as molecular pathology, genetics, forensics, immunopathology etc, will be attractive to non-medical scientists due to the shortage of medically qualified pathologists in these areas. The establishment of the Faculty of Science will look to the development of the future workforce thereby providing qualified expertise in specialist disciplines.

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Characterization of *Escherichia coli* and *Klebsiella pneumoniae* isolates for bla_{ESBLs}, bla_{ampC}, and bla_{NDM-1} from a north Indian tertiary hospital: a preliminary time trend study

Farrukh Sobia, Moin Uddin, Mohammad Shahid and Haris M Khan

Abstract

Background and aim: The global spread of antimicrobial resistant Gram-negative pathogenic organisms is considered as a major health concern as it leads to therapeutic failure. Moreover, transfer of resistance genes among bacteria results in augmentation of antibiotic resistance problem in pathogens. A constant surveillance is mandatory to know the resistance pattern; hence the incidence of beta-lactamase producing Enterobacterial strains (*Escherichia coli* and *Klebsiella pneumoniae*) was investigated and compared over a period of time.

Methods: Briefly, the study isolates were looked upon and compared for the occurrence of various *bla* genes in the three years of collection (91 isolates were collected in 2009, 34 in 2010, and 54 in 2012). bla_{NDM-1} was noticed in 27.78% isolates collected in the year 2012. These NDM-harboring isolates were also analyzed for their genetic environment.

Results and conclusions: The isolates showed association with insertion sequences suggesting them as major mobilizing genetic elements. Moreover, a small fraction showed presence of *Sul1*-type class 1 integron. When prevalence of *bla* genes was compared, bla_{ampC} showed a lining trend (80.22% to 76.47%, and then to 33.33%) in respective years. Whereas,occurrence of bla_{ESBLs} showed a somewhat different pattern. A slight increase was noticed in 2010, however, prevalence in 2012 was very similar to 2009. Surveillance of carbapenemase-producing organisms seems to be essential for proper implementation of infection control strategies and also for selection of appropriate antimicrobial therapy.

Key words: bla_{ESBLs}, bla_{ampC}, bla_{NDM-1}, mobile genetic elements, molecular survey.

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Introduction

The development of resistance to antimicrobial agents among Gram-negative pathogenic organisms has been progressive and inexorable. Of the few new drugs available, many have already become targets for bacterial resistance mechanisms. Pathogen of particular concern are the extended-spectrum β-lactamase (ESBL)-producing ones. ESBLs are plasmid-mediated β-lactamases which have an ability to hydrolyze β-lactam antibiotics containing an oxyimino group (e.g. ceftazidime, ceftriaxone, cefotaxime, or aztreonam). They have been most commonly found in *Klebsiella pneumoniae*, but are increasingly reported in *Escherichia coli*, *Proteus mirabilis*, and other members of Enterobacteriaceae.

ESBL enzymes initially come up through point mutations in the genes encoding the classic TEM and SHV β-lactamase, resulting in one or more amino acids substitution near the active site of enzyme thus increasing their affinity and hydrolytic activity against third generation cephalosporins and monobactams, and hence an array of oxyimino-β-lactam hydrolyzing enzymes with wider spectrum of activity has been generated. AmpC beta-lactamases are bacterial enzyme that confer resistance to oxyimino- and 7 α-methoxy cephalosporins and contribute to an appreciable resistance in the clinical isolates (1). In due course, various surveillance studies in different parts of the world have shown the predominance of TEM or SHV ESBLs, but in recent years, a new trend has been emerging i.e. the rapidly growing involvement of acquired AmpC beta-lactamase and CTX-M enzymes (2-4).

The genes encoding ESBLs and AmpC enzymes are located on plasmids which mediates their distribution. Recently, their mobilization through various insertion sequences and transposons have also been reported. The growing resistance rate in different microbial population can be attributed to these genetic vehicles which not only mediate their transfer in similar species but also results in inter-species relocation. Antibiotic resistance to a large extent is determined by acquisition of mobile genetic elements (MGEs). Such bundling of resistance genes on MGEs may lead to even faster acquisition of resistance genes. ESBL-producing organisms are also often found resistant to other non-beta-lactam antibiotics; it may be because of the presence of genes encoding such resistance mechanism on the same mobile genetic elements, viz. plasmids, integrons and transposons, along with genes for ESBLs (5).

Since the ESBL-producing organisms are posing major threat for clinical therapeutics, it is mandatory to identify the prevalence of such strains in hospitals and illustrate their epidemiology in order to control the spread of these strains by determining suitable preventive measures and treatment policies. Therefore, the present study was designed to characterize *E. coli* and *K. pneumoniae* isolates genotypically and also to compare the prevalence of resistance genes over a period of time.

Methods

Sample collection

A total of 125 isolates (109 *E. coli* and 16 *K. pneumoniae*) that were previously characterized for the presence of bla_{ampC} were looked for the co-existence of bla_{CTX-M}, bla_{TEM}, and bla_{SHV} (6). These isolates were categorized in two groups based on the period of collection of samples. Group I comprises of 91 isolates that were collected over a period of one year in 2009 and 34 isolates were included in group II, which were collected in the year 2010. Moreover, a subset of 54 isolates (37 *E. coli* and 17 *K. pneumoniae*) was collected after a gap of two year (in 2012) in order to compare the prevalence of bla_{ampC}, and bla_{ESBLs} in the respective years. The isolates of third group were

obtained from multiple sources including 37 from pus, 12 from urine, 2 from catheter tip, and one each from blood, drain, and peritoneal dialysis fluid.

Antimicrobial susceptibility testing
Antimicrobial susceptibility testing was performed by the Kirby-Bauer method and interpreted as per CLSI (formerly NCCLS) guidelines (7). *E. coli* ATCC 25922 was used as a control strain.

Detection of *bla*$_{ESBLs}$, *bla*$_{ampC}$, and *bla*$_{NDM-1}$
Crude genomic DNA was extracted from the isolates by heat lysis method. Briefly 2-3 bacterial colonies were suspended in 50 µL of molecular grade water and the cells were lysed by heating at 95°C for 5 min. and then immediately cooled to 4 ºC. The DNA was then screened for the presence of Class A ESBLs , more specifically, *bla*$_{CTXM}$, *bla*$_{TEM}$, and *bla*$_{SHV}$ by the PCR using the primers that span the universal region of CTX-M, TEM and SHV genes, as described previously (4). Presence of *bla*$_{NDM-1}$ was established by PCR using primer that specifically target NDM gene (8), whereas *bla*$_{ampC}$ was detected by the protocol of Feria *et al.* (2002) with some modifications (9).

Detection of mobilizing genetic elements (MGEs)
The study isolates were screened for various MGEs including insertion sequences (IS*Ecp1*, IS*26* and IS*CR1*) and *Sul-1*-type class1 integrons. Details of primers and size of amplified product are shown in Table 1.

Randomly amplified polymorphic DNA (RAPD) typing
RAPD-PCR typing of the *bla*$_{NDM-1}$-harboring isolates was done as described previously (10) to determine whether any specific clone is circulating in the hospital environment. Typing results were analyzed by using gel documentation system (BioRad) and clustering was done by Quantity One Software.

Results

Antimicrobial resistance pattern
Among Enterobacterial isolates collected in the year 2012, 50% (27/54) were found to be imipenem resistant. Moreover, resistance to aminoglycosides is still low as compared to cephalosporins and fluoroquinolones. Detailed antibiotic resistance patterns of the study isolates are shown in Table 2.

Table 1. Oligonucleotides used in the present study.

Target gene	Primer Sequence	Amplified product	Reference
bla$_{ampC}$	P1F: 5'-CCC CGC TTA TAG AGC AAC AA-3' P1R: 5'-TCA ATG GTC GAC TTC ACA CC-3'	634 bp	9
bla$_{CTX-M}$	P2F: 5'-ATG TGC AGY ACC AGT AAR GT 3' P2R: 5'-TGG GTR AAR TAR GTS ACC AGA 3' where Y, Wobble (C+T); R, Wobble (A+G) and S, Wobble (C+G)	593bp	4
bla$_{TEM}$	P3F: 5'-KAC AAT AAC CCT GRT AAA TGC-3' P3R: 5'-AGT ATA TAT GAG TAA ACT TGG 3' where K, Wobble (G+T) and R, Wobble (A+G)	936bp	4
bla$_{SHV}$	P4F: 5'-TTT ATC GGC CYT CAC TCA AGG-3' P4R: 5'-GCT GCG GGC CGG ATA ACG-3' where Y, Wobble (C+T)	930bp	4
bla$_{NDM-1}$	P5F: 5'-CAG CGC AGC TTG TCG-3' P5R: 5'-TCG CGA AGC TGA GCA-3'	800bp	8
IS*Ecp1*	P6F: 5'-AAA AAT GAT TGA AAG GTG GT-3' P6R: 5'-CAG CGC TTT TGC CGT CTA AG-3'	1100 bp	10
IS*26*	P7F:5'-GCG GTA AAT CGT GGA GTG AT-3' P7R: 5'-ATT CGG CAA GTT TTT GCT GT-3'	variable	10
IS*CR1*	P8F: 5'-CTC ACG CCC TGG CAA GGT TT-3' P8R: 5'-CTT TTG CCC TAG CTG CGG T-3'	600 bp	4
Sul1-type class 1 integron	P9F: 5'-CTT CGA TGA GAG CCG GCG GC-3' P9R: 5'-GCA AGG CGG AAA CCC GCG CC-3'	420 bp	4

Table 2. Antibiotic resistance rates in *E. coli* and *K. pneumoniae* isolates.

Antimicrobial agents (disc potency)	% Resistance (n=54)
Cefixime (5µg)	92.59 (50)
Ceftriaxone (30µg)	98.15 (53)
Cefotaxime (30µg)	90.74 (49)
Cefepime (30µg)	72.22 (39)
Gatifloxacin (5µg)	92.59 (50)
Ofloxacin (5µg)	96.30 (52)
Ciprofloxacin (5µg)	94.44 (51)
Gentamicin (10µg)	51.85 (28)
Amikacin (10µg)	48.15 (26)
Imipenem (10µg)	50.00 (27)

Figures in parentheses show number of resistant isolates.

Occurrence of various beta-lactamase genes

In the isolates collected in the year 2012, the maximum prevalence among ESBLs was noted to be that of bla_{CTX-M} (75.9%) followed by bla_{TEM} (61.1%) and then bla_{SHV} (44.4%) (Figure 1). Whereas, bla_{ampC} was found in 33.3% (18/54) isolates only (Figure 2). All the 18 AmpC-harboring isolates except one, were found to possess any of the Class A ESBL (CTX-M or TEM or SHV) gene or combination of more than one gene was also noticed. This clearly indicates their co-carriage on single plasmid or they are transferring through mobile genetic elements like insertion sequences and integrons. Surprisingly, we have noticed occurrence of bla_{NDM-1} in 27.8% (15/54) isolates that were collected in the year 2012 (Figure 3). Whereas, none of the isolates were found to harbor bla_{NDM-1} in the previous year's collection.

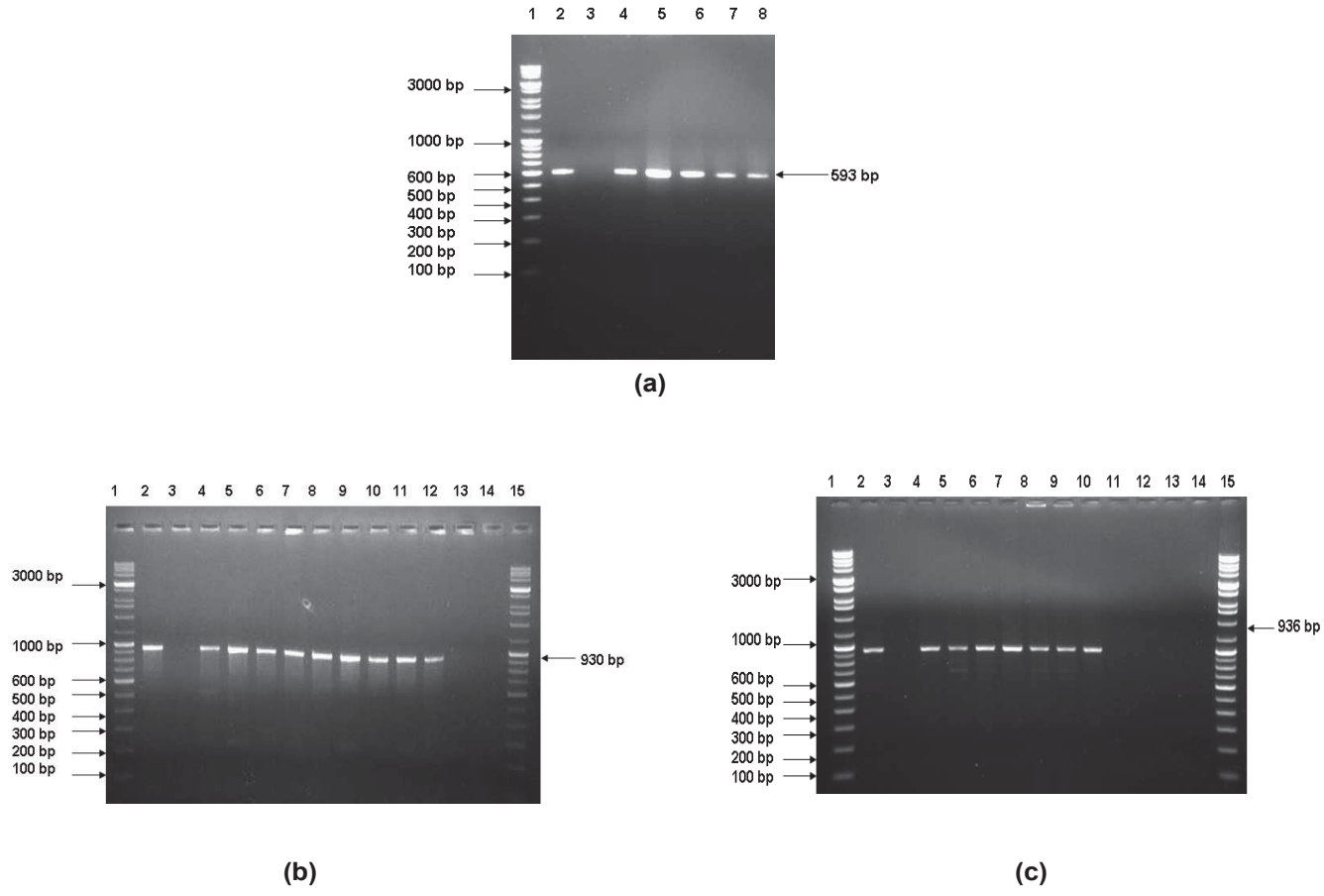

Figure 1.
(a): 2% agarose gel showing amplicons of bla_{CTX-M} where lanes 2 and 3 show positive and negative controls for bla_{CTX-M} (593 bp) respectively. Lanes 4 to 8 are positive clinical samples, and lane 1 is molecular weight marker (100 bp high range DNA ladder (Bangalore Genei, India).
(b): agarose (2%) gel shows amplification of bla_{SHV} (930 bp). Lanes 4 to 12 are clinical samples found positive for bla_{SHV}. Lanes 2 and 3 show positive and negative controls respectively, and lanes 1 and 15 are high range molecular weight markers (Bangalore Genei, India).
(c): 2% agarose gel showing bla_{TEM} amplicons (936 bp) and lanes 2 and 3 showed positive and negative control respectively. Lanes 4 to 10 are clinical samples positive for bla_{TEM}, and lanes 1 and 15 show molecular weight markers (Bangalore Genei, India).

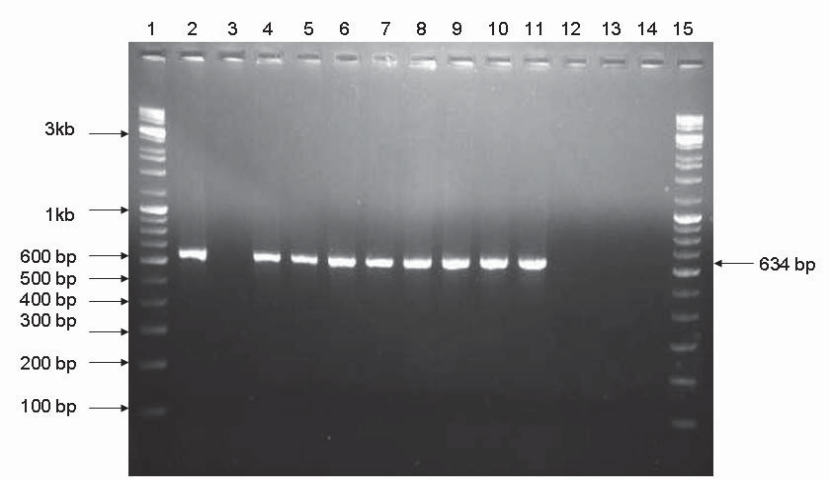

Figure 2. 2% agarose gel shows amplicons obtained for *bla*$_{ampC}$ gene, where Lanes 2 and 3 shows positive and negative control for *bla*$_{ampC}$ (634 bp) respectively. Lanes 4 to 11 are positive clinical samples, and lanes 12 to 14 are negative clinical isolates for ampC beta-lactamase. Lane 1 and 15 show molecular weight markers (100 bp high range DNA ladder (Bangalore Genei, India).

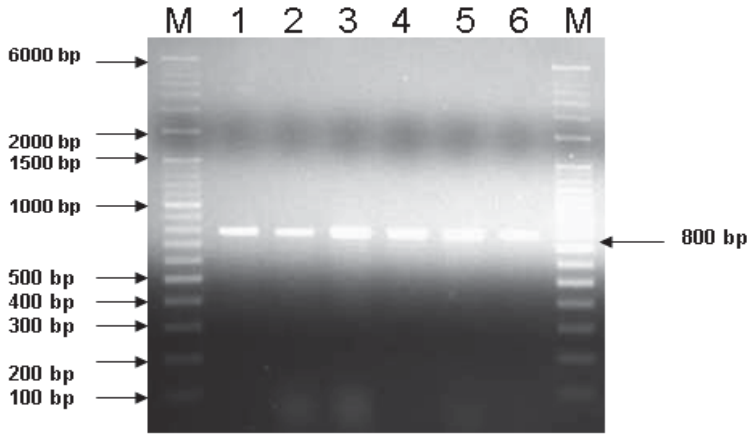

Figure 3. 2% agarose gel shows amplification of *bla*$_{NDM-1}$ (800 bp), where lanes marked as M shows molecular weight marker (Fermentas Life sciences, USA), lane 1 shows positive control and lanes 2 to 6 shows positive clinical samples.

Table 3. Association of NDM-1 harboring Enterobacterial isolates with various mobilizing genetic elements.

Combination of *bla* genes in *bla*$_{NDM-1}$-harboring isolates (n=15)	**Mobilizing genetic elements**	
	Insertion sequences	**Integron**
bla$_{CTX-M}$ + *bla*$_{ampC}$ + *bla*$_{TEM}$ + *bla*$_{NDM-1}$ (4/15)	IS*26* + IS*Ecp1* + IS*CR1* (2/4)	*Sul1*- type class 1 integron
	IS*Ecp1* + IS*CR1* (1/4)	*Sul1*- type class 1 integron
	IS*26* + IS*Ecp1* + IS*CR1* (1/4)	-
bla$_{CTX-M}$ + *bla*$_{ampC}$ + *bla*$_{TEM}$ + *bla*$_{SHV}$ + *bla*$_{NDM-1}$ (3/15)	IS*Ecp1* + IS*CR1* (3/3)	*Sul1*- type class 1 integron
bla$_{CTX-M}$ + *bla*$_{TEM}$ + *bla*$_{SHV}$ + *bla*$_{NDM-1}$ (3/15)	IS*26* + IS*Ecp1* (1/3)	*Sul1*- type class 1 integron
	IS*26* + IS*Ecp1* + IS*CR1* (1/3)	-
	IS*Ecp1* (1/3)	-
bla$_{CTX-M}$ + *bla*$_{TEM}$ + *bla*$_{NDM-1}$ (2/15)	IS*26* + IS*Ecp1* + IS*CR1* (2/2)	-
bla$_{CTX-M}$ + *bla*$_{ampC}$ + *bla*$_{NDM-1}$ (2/15)	IS*Ecp1* + IS*CR1* (1/2)	*Sul1*- type class 1 integron
	IS*26* + IS*CR1* (1/2)	-
bla$_{TEM}$ + *bla*$_{SHV}$ + *bla*$_{NDM-1}$ (1/15)	-	-

Analysis of *bla*$_{NDM-1}$-harboring isolates

As we observed that out of 15 NDM-1 positive isolates, only five showed the presence of *Sul-1*-type integrons indicating that probably in our study isolates, NDM-1 (along with combination of other *bla*$_{ESBLs}$) is mobilizing through insertion sequences and integrons is playing less role in their mobilization as compared to those of insertion sequences. Moreover, 12/15 NDM-harboring Enterobacterial isolates showed the presence of IS*CR1* indicating its probability as major mobilizing genetic element responsible for mobilization of this particular β-lactamase gene. Detailed genetic organization is shown in Table 3. Amplification pattern of various MGEs is shown in Figure 4.

RAPD analysis

Out of 15 NDM-1-harboring isolates, two (one each of *E. coli* and *K. pneumoniae*) were found untypable. However, diversity in bacterial population is still maintained and no particular clone was noticed among the study isolates. RAPD profile of *bla*$_{NDM-1}$-harboring *E. coli* and *K. pneumoniae* is shown in Figure 5.

Comparison of the Enterobacterial isolates for the presence of various *bla* genes in 2009, 2010 and 2012

When we compare the occurrence of AmpC and ESBLs in 2009, 2010 and 2012, we observed a slight decrease in *bla*$_{ampC}$ prevalence in the first two years (80.2% in 2009 to 76.5% in 2010) but after a gap of one year, a drastic decrease was noticed (33.3% in 2012). Among ESBLs, *bla*$_{CTX-M}$ prevalence was found to be 78.0% in 2009, increasing slightly to 88.2% in 2010, and dropping slightly to 75.9% in 2012. Occurrence of *bla*$_{TEM}$ and *bla*$_{SHV}$ showed more or less similar pattern, with the occurrence of *bla*$_{TEM}$ almost identical in 2010 and 2012.

Prevalence of *bla*$_{TEM}$ was noted to be 37.4% in 2009, increased to 64.7% in 2010 and then decreased slightly to 61.1% in 2012. Moreover, the occurrence of *bla*$_{SHV}$ was noticed to be 38.5% in 2009, it then increased to 76.5% in 2010 but a significant decrease was noticed in 2012 with a 44.4% prevalence rate. Occurrence of *bla*$_{NDM-1}$ was noticed only in the isolates collected in 2012. Comparative trends can be seen in Figure 6.

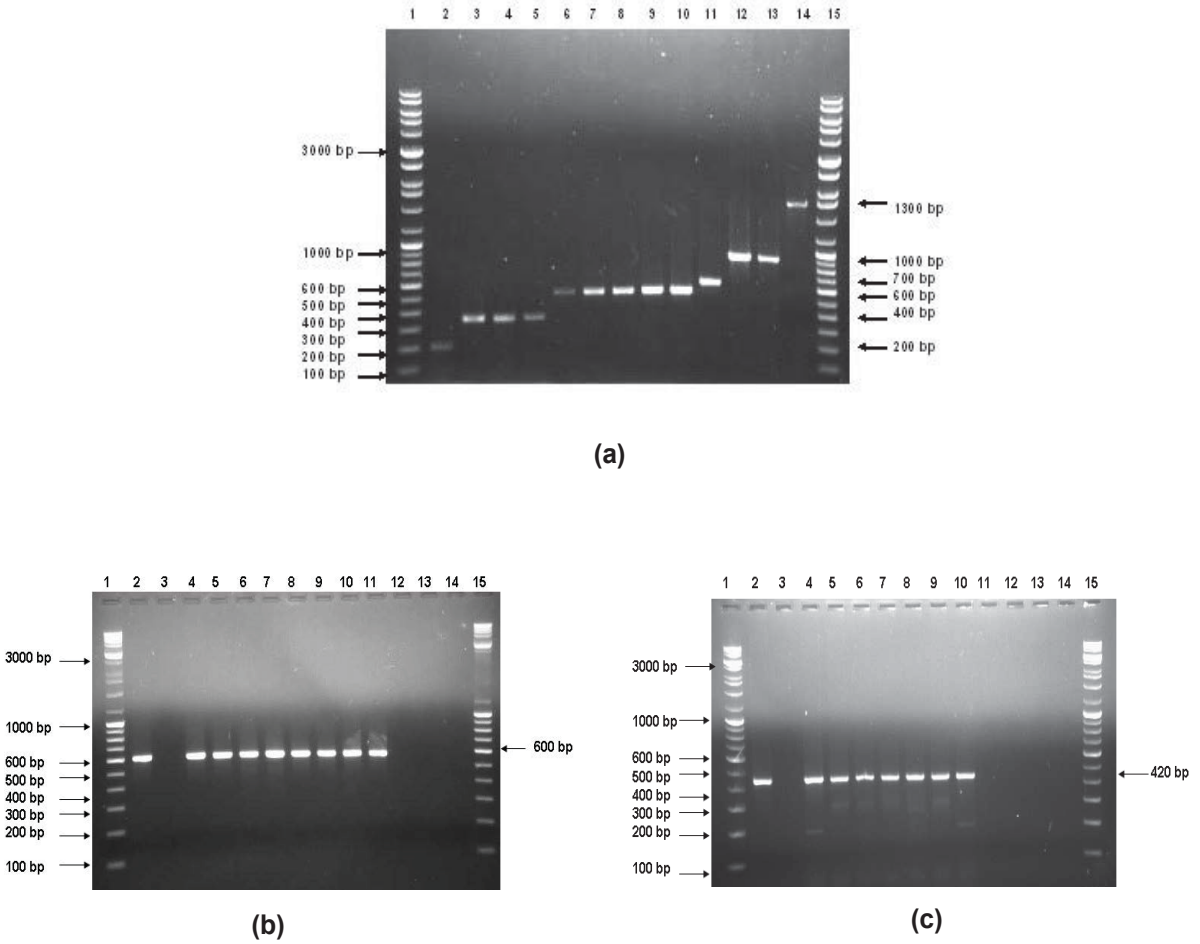

Figure 4.

(a): Agarose gel (2%) shows amplicons obtained for IS*26* insertions. Lanes 1 and 15 shows molecular weight markers (High range DNA Ladder; Bangalore Genei, India). Lanes 2 to 14 shows the amplified products of varying molecular weight noticed in clinical samples.

(b): Agarose gel (2.0%) shows amplicons of IS*CR1* (600 bp). Molecular weight marker (High range DNA Ladder; Bangalore Genei, India) is shown in lanes 1 and 15. Lanes 2 and 3 shows positive and negative control strains respectively, whereas lanes 4 to 11 shows amplicons from clinical isolates. Lanes 13 and 14 shows clinical samples found negative for the respective gene.

(c): agarose (2%) gel shows amplification results of *Sul-1* gene (420 bp). Lanes 1 and 15 shows high range DNA ruler (Bangalore Genei, India). Lanes 2 and 3 shows positive and negative controls respectively, while lanes 4 to 10 shows clinical samples positive for *Sul-1* gene.

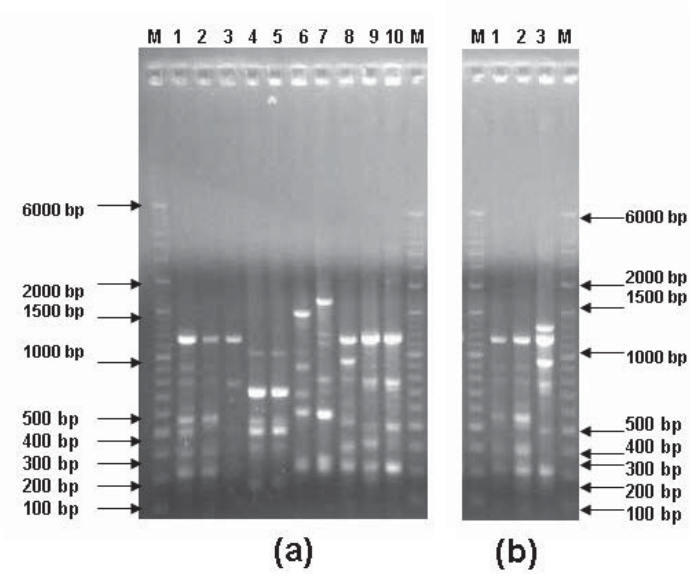

Figure 5.
(a): 2% agarose gel shows RAPD profile of *bla*$_{NDM-1}$-harboring *E. coli* isolates, whereas lanes marked as M shows molecular weight marker (Fermentas Life sciences, USA).
(b): Agarose gel (2%) shows RAPD profile of *Klebsiella pneumoniae* isolates that possess NDM-1 β-lactamase. Lanes M shows high range molecular weight marker (Fermentas Life Sciences, USA).

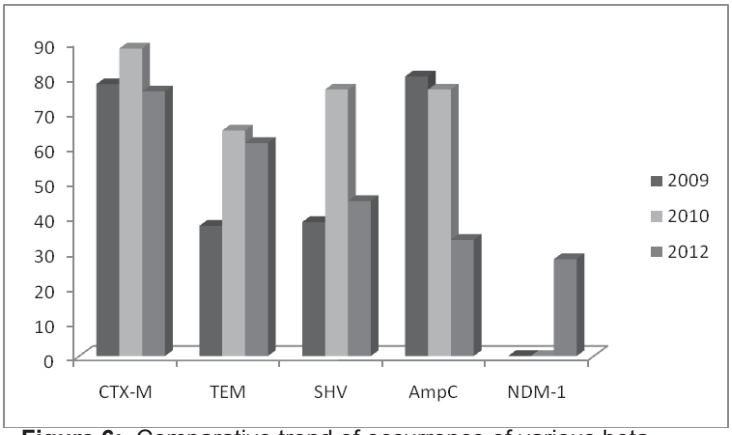

Figure 6: Comparative trend of occurrence of various beta-lactamase genes.

Discussion

Emerging bacterial resistance is the most serious problem being faced in the clinical practice today. The term ESBLs was originally applied to describe the TEM and SHV variants that can hydrolyze oxyiminocephalosporins (11). The extended-spectrum of activity can be defined in terms of hydrolysis of oxyiminocephalosporins or aztreonam at >10% of the activity of hydrolyzing benzylpenicillins. Generally they confer resistance to 1st, 2nd and 3rd generation cephalosporins along with monobactams, but are sensitive to cephamycins and carbapenems (12). Most of ESBls-coding genes are plasmid-borne but can be located on transposons and integrons (13) that facilitate their mobilization. CTX-M type of ESBL was reported in 1989 and is now considered as one of the most dominant type of ESBL in many countries. AmpC beta-lactamases are clinically important cephalosporinases, confer resistance to cephalothin, cefazolin, cefoxitin, most penicillins and β-lactam-β-lactamase inhibitor combinations.

Reports on the prevalence of ESBLs in India have been recorded since the 1990s (14). Jemima and Verghese reported a prevalence of *bla*$_{CTX-M-1}$ in 15.8% isolates from the southern part of India (15). In the southern part of India, prevalence of ESBLs was reported to be 40% (16). Moreover, Anandan *et al*.reported 80% *Klebsiella* spp. and 63.6% *E. coli* as ESBL-producers in pediatric patients with septicemia (17). Recently, in a study performed in Rajasthan by Dalela, 73.5% *E. coli* and 58.1% *Klebsiella pneumoniae* isolates were reported as ESBL producers (18).

However, we isolated 78.0% CTX-M-producers in 2009, slightly increased to 88.2% in 2010, and then in 2012 the occurrence of *bla*$_{CTX-M}$ was very similar to that in 2009. Moreover, TEM-producers were found to be 37.4% in 2009, increased to 64.7% in 2010 and 61.1% in 2012. Prevalence of *bla*$_{SHV}$ was noticed to be 38.5% in 2009, 76.5% in 2010 and 44.4% in 2012.

Manoharan *et al*. reported 36.5% cefoxitin-resistant isolates as AmpC-producers in a study conducted on isolates taken from five Indian medical centers (19). However, in our study isolates, occurrence of *bla*$_{ampC}$ was found to be 80.2% in the year 2009, decreased to 76.5% in 2010 and a further decrease was observed in the 2012 with a prevalence of 33.3%.

Resistance to carbapenem is often mediated by production of MBL, a class B-type beta-lactamases that requires bivalent metal ion, usually Zn for their activity. NDM-1 share only 32.4% amino acid sequence homology with the closely related MBLs (VIM-1/VIM-2), and transpose hastily to other organisms via rolling circle mechanism facilitated by IS*CR1* (20). NDM has been originally identified in a *K. pneumoniae* isolate from a Swedish patient of Indian origin, has now been reported in isolates worldwide among Gram-negative bacterial species (20-23). A study from Pakistan reported occurrence of *bla*$_{NDM-1}$ in 27.1% of inpatients and 13.8% of outpatients (24). We also noticed occurrence of *bla*$_{NDM-1}$ in almost similar fraction (27.8%) of our Enterobacterial isolates.

In addition to Enterobacteriaceae, *bla*$_{NDM-1}$ has also been reported in non-fermentative bacteria like *Acinetobacter* spp. (21). NDM-1 β-lactamases has also been reported in Hong Kong and Taiwan in *E. coli* and *K. pneumoniae* isolates (25,26). Co-carriage of *bla*$_{NDM-1}$ and *bla*$_{ESBLs}$, more specifically *bla*$_{CTX-M-15}$ and *bla*$_{CTX-M-14}$ has also been reported (23,27). Recently, a novel variant of NDM (NDM-8) has been reported by Tada *et al*. in a multidrug-resistant *E. coli* isolate (28). Furthermore, 1.0% of Enterobacterial isolates from Indian hospitals were reported to harbour *bla*$_{NDM-1}$ (29). Kumarasamy *et al*. reported prevalence of NDM-1 in 1.2% enterobacterial isolates from Chennai and 13.1% isolates from Haryana (30). Shweta *et al.* have recently reported occurrence of *bla*$_{NDM-1}$ in 3 (out of 74) *Acinetobacter baumanii* isolates from a tertiary care hospital of Northern India (31). They also reported co-production of EBC, DHA, and CIT AmpC families in all the three NDM-harboring isolates. It has been reported that *bla*$_{NDM}$ is closely associated with IS*CR1* and IS*CR16* and is supposed to get mobilized through these insertion sequences. However some studies showed its mobilization through IS*Aba125* sequences (31). We also observed association of *bla*$_{NDM-1}$ with IS*CR1* rather than *Sul-1*-type class 1 integrons in our bacterial population; however, it is in contrast to the report of Farzana *et al*. who reported that all NDM-1-harbouring isolates (8/31) were associated with class 1 integrons (32).

The free exchange of genetic material across the bacterial world ensures the survival of infectious agents. It is a well known fact that the resistance determinants were present in bacterial population before the therapeutic use of antibiotics; the current widespread prevalence is certainly due to selection pressure generated by human activity. Furthermore, conjugational transfer of antibiotic resistance genes across bacterial species and genera has amplified the problem of antibiotic resistance among pathogenic organisms. It has also been reported that *bla*$_{NDM-1}$ harboring plasmid, also possess an array of co-resistance determinants including various β-lactamase genes, genes responsible for quinolone resistance etc. (33).

Regular surveillance of antimicrobial drug resistance is highly recommended. In the present study the change in such a trend, i.e. an initial increase followed by a decrease could have

resulted from (i) inconsistency in the sample collection time, (ii) inconsistent sample size, and (iii) may be due to implementation of a strict antibiotic policy at our institution following a few preceding publications in this regard. As we noticed wide provincial differences, so it seems necessary to take into account the local epidemiology; more specifically at the level of country, particular region, and even particular hospitals. It further helps in making decisions about empirical therapy. Moreover, proper application of surveillance is essential to reduce current drug resistance rate in hospitals as well as in communities. Surveillance of carbapenemase-producing organisms seems to be essential for proper implementation of infection control strategies and also for selection of appropriate antimicrobial therapy. Moreover, infection control measures will be key factor in minimizing spread of NDM-1-harboring plasmids in hospital settings.

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Interference of spurious haemolysis on prothrombin time, activated partial thromboplastin time, and fibrinogen

Giuseppe Lippi and Luigi Ippolito

Abstract

Objectives: A number of laboratory tests are substantially biased by spurious haemolysis. However, there is still controversy around the impact of this issue on routine coagulation studies. As such, we planned an investigation aimed to establish whether sample haemolysis may generate a different bias in samples collected from healthy subjects or in patients undergoing warfarin therapy.

Methods: The study population consisted of 24 subjects, 12 undergoing warfarin therapy and 12 healthy subjects not taking any medications. Whole blood was divided in four aliquots. The first aliquot was immediately centrifuged and tested, whereas the remaining were subjected to mechanical haemolysis by 1-, 2- and 3-time aspiration through a fine needle, then centrifuged and assayed for prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen.

Results: The concentration of cell-free hemoglobin increased from 0.35±0.16 g/L in baseline samples to 2.40±0.85 g/L in 1-time haemolyzed aliquots, 2.89±0.63 g/L in 2-time haemolyzed aliquots, up to 3.62±1.87 g/L in 3-time haemolyzed aliquots. The values of all coagulation parameters remained unchanged in haemolyzed aliquots of both healthy subjects and patients on warfarin therapy, with no significant difference compared to the baseline aliquot without haemolysis. The maximum bias that could be recorded was within the desirable specifications for total error.

Conclusions: Results of PT, APTT and fibrinogen in patient samples containing up to 3.6 g/L of cell-free hemoglobin are reliable regardless of their origin and do not require to be suppressed, nor samples need to be recollected.

Key words: interference, pre-analytical variability, haemolysis, quality.

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Introduction

The results of laboratory testing provide an essential contribution to the clinical decision making, provided that a high degree of quality can be assured throughout the testing process. There is now consolidated evidence that the greatest vulnerability of diagnostic testing emerges from manually intensive activities of the preanalytical phase and, especially, to those steps related to drawing and handling of blood samples (1). More specifically, the collection of diagnostic blood specimens by venipuncture is conventionally regarded as a high risk activity that may impair the reliability of testing, since the quality of samples may be jeopardized by contamination by infusion fluids (2), or be biased by blood cell injury (3). This latter process, conventionally known as “spurious” or “in vitro” haemolysis, is the leading source of errors throughout the total testing process, since the analysis of frankly haemolyzed samples may generate a substantial bias in test results, which would finally contribute to impair the clinical reasoning and potentially jeopardize patient safety (4).

It is now widely accepted that certain tests such as glucose, lactate dehydrogenase (LDH), potassium (5), as well as cardiospecific troponins (6), should be systematically suppressed in samples with significant degree of haemolysis (i.e., concentration of cell-free hemoglobin greater than 0.5-0.6 g/L). Nonetheless, there is still controversy around the impact of spurious haemolysis on routine and specialized coagulation testing. It has been conventionally recommended to maintain a cautionary approach dealing with this issue, which is sample recollection whenever the amount of cell-free hemoglobin in citrated plasma exceeds a certain concentration (typically between 2 and 3 g/L) (7). Nevertheless, recent and convincing evidence has been provided that a different strategy may be advisable between healthy subjects and patients undergoing anticoagulant therapy (8), since test results obtained in the former population may be less vulnerable to blood cell injury. As such, in order to verify this hypothesis, we planned a specific study aimed to establish whether sample haemolysis may generate a different bias in samples collected from healthy subjects or in patients undergoing stable warfarin therapy.

Materials and methods

The study population consisted of 24 subjects, randomly selected from those referred to our outpatient phlebotomy center for routine coagulation testing over the same working day. Twelve patients were undergoing stable anticoagulant therapy with warfarin, whereas the remaining were healthy subjects not taking any medications and undergoing a routine preoperative screening. All patient samples were collected in 2.7 mL BD Vacutainer plastic tubes containing 0.109 mol/L buffered sodium citrate (Becton Dickinson Italia, Milan, Italy). According to our study design, routine analysis was performed as for local practice.

After all routine tests had been completed, the centrifuged samples were re-suspended by gentle inversion of the primary blood tubes and then divided in four aliquots of ~0.6 mL each. The first aliquot was immediately centrifuged and tested. The remaining three aliquots of each patient sample were subjected to mechanical haemolysis by one (i.e., 1-time haemolyzed aliquot), two (i.e., 2-time haemolyzed aliquot), and three times (i.e., 3-time haemolyzed aliquot) aspiration through a fine needle (30 gauge, 0.3x8 mm) connected with an insulin syringe (Picindolor, Artsana S.p.A., Grandate, Italy). This procedure allows us to obtain different amounts of spurious haemolysis, as described elsewhere (9). These aliquots were then centrifuged and analyzed as described below. The amount of cell-free hemoglobin was quantified by a spectrophotometric technique on a Beckman Coulter Unicel DxC (Beckman Coulter Inc., Brea, CA, USA).

Coagulation studies on the samples included prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen, which were assessed on an ACL TOP 700 (Instrumentation Laboratory Company, Bedford, MA, USA), using RecombiPlasTin, SynthASil and FibrinogenC-XL, respectively. The inter-assay imprecision of these tests has been reported to be <1.0% for RecombiPlasTin, <2.0% for SynthASil and <7.7% for Fibrinogen C-XL, respectively (10).

The results of measurements in the baseline aliquots and in those containing increasing levels of cell-free hemoglobin were evaluated by Student’s paired T test and direct comparison with the desirable specifications for total error, as recommended by Ricos et al (11). Results of coagulation tests are reported as median and interquartile range (IQR). The statistical analysis

was performed with Analyse-it (Analyse-it Software Ltd, Leeds, UK). The experiments were based on pre-existing samples, thus informed consent or approval by an ethics committee was unnecessary. The study was performed in accordance with the Declaration of Helsinki and under the terms of all relevant local legislations.

Results

The mean concentration of cell-free hemoglobin in the different aliquots increased from 0.35±0.16 g/L in the baseline samples, to 2.40±0.85 g/L in the first haemolyzed aliquots, 2.89±0.63 g/L in the second haemolyzed aliquots, and up to 3.62±1.87 g/L in the third haemolyzed aliquots (Table 1).

Table 1. Interference of spurious haemolysis on prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen in samples collected from healthy subjects (n=12) and in patients undergoing stable warfarin therapy (n=12). Results are shown as median and interquartile range (IQR).

	Baseline samples	One-time haemolyzed aliquots	Two-time haemolyzed aliquots	Three-time haemolyzed aliquots
Cell-free hemoglobin (g/L)	0.35±0.16	2.40±0.85†	2.89±0.63†	3.62±1.87†
PT (s)				
- Healthy subjects	10.9 (8.7-14.4)	11.0 (8.8-14.2)	11.0 (8.8-14.4)	11.1 (8.9-14.5)
- Patients on warfarin	30.3 (29.0-32.6)	29.8 (28.5-31.9)	29.7 (28.6-31.7)	29.6 (28.4-31.8)
APTT (s)				
- Healthy subjects	29.7 (28.7-30.7)	29.3 (28.5-30.0)	29.9 (29.0-31.1)	29.8 (28.9-30.7)
- Patients on warfarin	37.9 (34.7-43.6)	36.8 (33.6-42.9)	37.1 (34.1-42.7)	37.6 (34.3-43.2)
Fibrinogen (mg/dL)				
- Healthy subjects	577 (535-624)	553 (517-612)	559 (521-609)	559 (520-609)
- Patients on warfarin	391 (362-428)	391 (426-360)	392 (425-360)	382 (352-422)

† p>0.01 compared to baseline sample.

The values of all coagulation parameters remained substantially unchanged in the haemolyzed aliquots of both healthy subjects and patients on warfarin therapy. More specifically, no significant differences could be observed in haemolyzed samples compared with the baseline aliquots with no haemolysis for any of the parameters tested (Table 1; p>0.05 for all). The maximum bias was comprised between –2.3% and +1.8% for PT, -2.9% and +0.7% for APTT, and -4.0% and -0.1% for fibrinogen, and were within the desirable specifications for total error (±5.3% for PT, ±4.5% for APTT and ±13.6% for fibrinogen) (11).

Discussion

Laboratory errors occur at a rate that is no different to any complex industry. Although the largest part of these errors has little to null consequence on patient health because laboratory systems are typically built around a paradigm of quality, a minority of them, when not prevented or appropriately intercepted, may still generate serious harm (12).

Sample haemolysis is probably the greatest challenge to the reliability of diagnostic testing. The suppression of tests results, which may be necessary whenever laboratory data are frankly biased by haemolysis, causes diagnostic delays and raises a number of economic and organizational issues between the laboratory and the wards. On the other hand, the transmission of unreliable data to the requesting physicians may seriously jeopardize patient safety (4). It is hence reasonable to suggest that a comprehensive evaluation of haemolysis interference should be regarded as a cornerstone for planning the most appropriate strategy for managing haemolyzed samples.

In a recent article, Arora *et al.* concluded that samples referred to the laboratory for routine coagulation studies and displaying visible haemolysis may be suitable for testing (8). This is an important message, which was also confirmed in our study. In particular, in agreement with data of Arora *et al*., we observed a very modest prolongation of the PT and an analogously modest shortening of the APTT, especially in samples taken from patients undergoing warfarin therapy. In both cases, however, the percentage bias was substantially lower than the desirable specifications for total error. In addiction to these findings, we also measured fibrinogen in these samples, and we observed that neither this parameter is significantly biased by spurious haemolysis. At variance with Arora et al, we directly measured the concentration of cell-free hemoglobin by a spectrophotometric technique, which allowed us to clearly establish the concentration of this parameter in the specimens.

It is also noteworthy that a significant difference was found in the results of this study compared to a previous investigation, in which significant increase in PT and significant decreases in APTT and fibrinogen were observed with moderate lysis (13). However, the two study designs were different, since in the present study we used a different approach to test the haemolysis interference on routine clotting tests (i.e., mechanical haemolysis of whole citrated blood versus spiking hemolysate) (14), the range of final concentration of cell-free hemoglobin was nearly one fourth lower than in the previous study (0-4.4 g/L versus 0-17 g/L), and different instrumentation and reagents were used (i.e., IL ACL Top versus Dade Behring Coagulation System). This last aspect identifies an important point in that each laboratory should perform a verification of bias, to identify the most reliable thresholds of haemolysis interference according to local reagents and instrumentation.

Conclusions

The results of this study enabled us to define that samples containing up to 3.6 g/L of cell-free hemoglobin, which represent approximately the vast majority of the haemolyzed specimens received by clinical laboratories (3), may be analyzed regardless of their source (i.e., healthy subjects or patients undergoing warfarin therapy), thus avoiding diagnostic delays and problems between the laboratory and short stay units such as the emergency department and the intensive care unit. Another aspect that deserves a special mention is the practicability of using the automated assessment of cell-free hemoglobin concentration in serum or plasma samples, especially in those that display visible haemolysis. Its measurement by means of the haemolysis index, that also has become available on coagulation analyzers (15), was proven to be reliable and practical (16), so that this practice should be encouraged to provide an objective and virtually incontestable measure of sample quality.

A limitation is that this is a pilot study and these findings should be confirmed using a larger sample size, and especially, in patients taking heparin, or oral anticoagulants such as dabigatran, rivaroxaban, apixaban or edoxaban (17).

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Uptake of HIV, HBV and HCV testing services among medical laboratory scientists in Nigeria

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Abstract

Objectives: Medical laboratory workers are at occupational risk of contracting blood borne viral diseases. Early detection of disease is critical for effective management and care. Against this background, this study aimed at assessing the frequency and factors associated with uptake of human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) testing services among medical laboratory scientists working in medical diagnostic laboratories in Nigeria

Methods: A total of 110 medical laboratory scientists (consisting of 31 in public and 79 in private diagnostic laboratories) were recruited for this study. A detailed questionnaire was used to obtain relevant information from the study participants.

Results: All participants reported to have had at least one post qualification HIV screening test. However, only 41 (37.3%) and 17 (15.5%) of participants claimed to have been screened for HBV and HCV respectively. HIV, HBV and HCV testing in the previous twelve months was reported by 65 (59.1%), 12 (10.9%), and 8 (7.2%) of study participants respectively. HIV testing in the previous12 months was significantly affected by gender (female vs. male: 69.6% vs 41.5%; OR = 3.23, 95% CI = 1.44 - 7.22, P =0.005) and post qualification experience (P = 0.020) of study participants. Only a history of blood donation was observed to significantly affect HCV testing in the previous twelve months among study participants (OR = 5.42, 95% CI = 1.21 - 24.35, P = 0.028). None of the demographic factors considered in this study were observed to significantly affect HBV testing in the last 12 months.

Conclusions: Uptake of HBV and HCV testing services among medical laboratory scientists was poor. Regular screening for HIV, HBV and HCV is advocated among medical laboratory scientists in Nigeria.

Key words: HBV, HCV, HIV, medical laboratory scientists, Nigeria, routine testing.

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Introduction

Health care workers (HCWs) are at an increased risk of contracting blood borne viral diseases (1). Accidental needle-stick or sharps injuries caused by hollow bore needles or other objects constitutes an important occupational health hazard for health care professionals and provides the most important route of infection for human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) (2). Occupationally related HIV infection among HCWs exposed to blood and body fluids has been previously documented (3,4,5). In Botswana, an estimated 17% of the health workforce was lost to HIV infection from 1999 to 2005 (6). Data from Zimbabwe, Mozambique, Malawi, Kenya and Ethiopia indicate that 43% of deaths and medical retirements were suspected or known to be caused by HIV infection (7).Reports indicate that about 2.5% of HIV cases and 40% of all HBV and HCV cases among HCWs worldwide are the result of occupational exposure (8).The risk of contracting HBV infection by HCWs is reported to be four-times greater than that of general adult population (1). HCV transmission following needle-stick injury is also a recognized threat to HCWs (9).

The medical laboratory scientist occupies a prime position in the fight against diseases, as this group is actively involved in the analysis of human and biological fluids and tissues for the purpose of medical diagnosis, treatment and research. Medical laboratory scientists undertake the testing of clinical samples from high risk patients and are at risk of contracting blood borne diseases. Although international recommendations exist for promoting the safety of the laboratory personnel in the work place (10), reports from recent Nigerian studies indicates that adherence to such guidelines is poor (11-13). The occupational risk of HIV infection among HCWs is reported to depend on the population prevalence of HIV infection, type of injury sustained and the response to laboratory acquired injuries (14).

Nigeria is Africa's most populous nation and home to more people living with HIV than any other country in the world, except for South Africa and India (15). Furthermore, a previous Nigerian study has also reported very poor disease prevention knowledge among laboratory workers following occupational exposure to potentially infectious blood and body fluids (12). Laboratory personnel working routinely with samples of human origin are likely to have an increased risk of contracting blood borne viral diseases in Nigeria.

The Center for Disease Control, in its revised guidelines for HIV testing in adults, adolescents and pregnant women in health care settings, recommends that all persons at increased risk of occupational HIV infection be screened at least once annually (16).There is a high rate of health worker attrition in Sub-Saharan Africa, attributable to HIV infection (17). This implies a low uptake infectious disease testing among HCWs (17). HCWs usually know where to access HIV testing services, which indeed are often found at their own workplaces; however, this knowledge is often not enough to promote regular HIV testing among HCWs (17). As a consequence of shared routes of transmission, HBV and HCV infection are common among persons infected with HIV (18,19). While guidelines for HBV testing and vaccination have been in place since the early days of the HIV epidemic, management of chronic HBV infection has been inconsistent and marked by the failure to recognize and adequately treat persons with active HBV infection-related liver disease (18). Early detection of acute HCV infection is critical and should prompt the consideration of anti-HCV therapy to prevent chronic HCV infection (18). There is presently no vaccine for the prevention of HCV infection, making early detection of infection a critical aspect of HCV control measures. Although actively involved in offering valuable HIV, HBV and HCV screening services for the general public and patients, nothing is known about the uptake of HIV, HBV and HCV testing services among medical laboratory scientists in Nigeria. Against this background, this study set out to assess the regularity of occupational HIV, HBV and HCV testing and factors affecting this, among medical laboratory scientists working in medical diagnostic laboratories in Nigeria.

Materials and Methods

Study population

A total of 121 medical laboratory scientists were contacted for this study and 110 (90.9%) agreed to participate. They were drawn from different health facilities spread across rural and urban settlements in Edo State, Nigeria. All participating laboratory personnel had at least one year of post qualification laboratory work experience. An anonymous questionnaire (Appendix at end of article) was used to obtain relevant information from study participants such as gender, area of specialization location of work place (urban or rural), institutional affiliation (public or private), years of post-qualification experience, history of blood donation, and HIV, HBV and HCV testing experience. Informed consent was obtained from all participants prior to administration of the questionnaire. Study approval was obtained from the Edo State Ministry of Health, Benin City, Nigeria.

Statistical analysis

The data obtained were analyzed using Chi square (X^2) test and odds ratio analysis using the statistical software INSTAT® (Graphpad software Inc., La Jolla, CA, USA). Statistical significance was set at $P < 0.05$.

Results

All participants reported to have had at least one post-qualification HIV status screening test. However, only 41(37.3%) and 17 (15.5%) of study participants reported to have been screened for HBV and HCV respectively. HIV, HBV and HCV status testing in the last twelve months was observed in 65(59.1%), 12 (10.9%), and 8 (7.2%) of study participants respectively.

HIV testing in the previous 12 months was significantly affected by gender and post qualification experience of study participants (Table 2). HBV status testing in last twelve months was not significantly affected by gender area of specialisation, affiliation, location of work place, post qualification experience, or history of blood donation (Table 3). Only a previous history of blood donation significantly affected HCV testing in the previous twelve months (Table 4). None of the demographic factors considered in this study significantly affected HBV testing in last 12 months.

Table 1. Post qualification testing rates for HIV, HBV and HCV among medical laboratory scientists (n=110).

Variable	Yes (%)
Ever had post qualification HIV screening	110 (100%)
Ever had post qualification HBV testing	41 (37.3%)
Ever had post qualification HCV testing	17 (15.5%)

Table 2. Factors associated with HIV testing in the previous 12 months among medical laboratory scientists.

Variable	N	Tested for HIV (%)	OR	95% CI	P
Gender					
Male	41	17 (41.5)	0.31	0.14 - 0.69	
Female	69	48 (69.6)	3.23	1.44 - 7.22	0.005
Area of specialisation					
Haematology	17	12 (70.5)			
Medical microbiology	55	34 (61.8)			
Chemical pathology	28	13 (46.4)			
Histopathology	10	6 (60.0)			0.088
Affiliation					
Public laboratory	31	16 (51.6)	0.65	0.28 - 1.51	
Private laboratory	79	49 (62.0)	1.53	0.66 - 3.54	0.389
Location of work place					
Urban	93	59 (62.3)	2.46	0.81 - 7.48	
Rural	17	6 (41.2)	0.41	0.66 – 3.54	0.156
Post-qualification experience					
1-3 years	33	24 (72.7)			
4-6 years	51	30 (58.8)			
7-9 years	16	7 (43.8)			
≥ 10 years	10	4 (40.0)			0.020
Ever donated blood					
Yes	29	16 (33.1)	0.80	0.34 – 1.89	
No	81	49 (60.5)	1.24	0.53 – 2.93	0.664

OR = odds ratio. CI = confidence interval.

Table 3. Factors associated with HBV testing in the previous 12 months among medical laboratory scientists.

Variable	N	Tested for HIV (%)	OR	95% CI	P
Gender					
Male	41	6 (14.6)	1.80	0.54 – 6.01	
Female	69	6 (8.7)	0.56	0.17 – 1.85	0.358
Area of specialisation					
Haematology	17	4 (23.5)			
Medical microbiology	55	5 (9.1)			
Chemical pathology	28	3 (10.7)			
Histopathology	10	0 (0.0)			0.241
Affiliation					
Public laboratory	31	6 (19.4)	2.92	0.86 – 9.89	
Private laboratory	79	6 (7.6)	0.34	0.10 – 1.16	0.094
Location of work place					
Urban	93	9 (9.6)	0.50	0.12 – 2.08	
Rural	17	3 (17.6)	2.00	0.48 – 8.31	0.393
Post-qualification experience					
1-3 years	33	3 (9.1)			
4-6 years	51	6 (11.7)			
7-9 years	16	2 (12.5)			
≥ 10 years	10	1 (10.0)			0.819
Ever donated blood					
Yes	29	3 (10.3)	0.92	0.23 – 3.68	
No	81	9 (11.1)	1.08	0.27 – 4.31	1.000

OR = odds ratio. CI = confidence interval.

Table 4. Factors associated with HCV testing in the previous 12 months among medical laboratory scientists.

Variable	N	Tested for HIV (%)	OR	95% CI	P
Gender					
Male	41	4 (9.7)	1.38	0.35 – 5.48	
Female	69	5 (7.2)	0.72	0.18 – 2.86	0.725
Area of specialisation					
Haematology	17	2 (11.7)			
Medical microbiology	55	3 (5.5)			
Chemical pathology	28	2 (7.1)			
Histopathology	10	1 (10.0)			0.828
Affiliation					
Public laboratory	31	2 (6.5)	0.84	0.16 – 4.40	
Private laboratory	79	6 (7.5)	1.19	0.23 – 6.25	1.000
Location of work place					
Urban	93	5 (5.4)	0.27	0.06 – 1.24	
Rural	17	3 (17.6)	3.77	0.81 – 17.57	0.105
Post-qualification experience					
1-3 years	33	3 (9.1)			
4-6 years	51	4 (7.8)			
7-9 years	16	2 (12.5)			
≥ 10 years	10	0 (0.0)			0.629
Ever donated blood					
Yes	29	5 (17.2)	5.42	1.21 – 24.35	
No	81	3 (3.7)	0.19	0.04 – 0.83	0.028

OR = odds ratio. CI = confidence interval.

Discussion

The Center for Disease Control, in its revised guidelines for HIV testing in adults, adolescents and pregnant women in health care settings, recommends that all persons at high risk of HIV infection be screened at least once annually (16). HCWs are at risk of acquiring blood-borne viral infections, particularly HBV, HCV, and HIV (20). This is particularly prevalent in regions known to be endemic for HIV, HBV and HCV infections such as sub-Saharan Africa (20). Against this background, this study assessed HIV, HBV and HCV status testing rates among medical laboratory scientists working in medical diagnostic laboratories in Nigeria. To our knowledge, this is the first study of HIV, HBV and HCV testing rates among medical laboratory scientists.

All medical laboratory scientists recruited in this study reported to have had at least one post-qualification HIV testing. This is higher than the 86.5% reported among medical laboratory technicians in Cameroonian (20). However, only 65(59.1%) of our study participants reported to have been tested at least once for HIV in the last 12 months. This rate is slightly lower than the 64.8% reported among HCWs in another African study (21). Medical laboratory workers in sub-Saharan Africa can be expected to have increased contact with HIV infected individuals and their specimens, placing them at an increased risk of occupational HIV infection. Randomized and controlled trials have shown that there is a lower risk of death or AIDS defining illness in HIV – infected persons when highly active anti-retroviral therapy (HAART) is initiated with CD4+ counts between 350 and 500 cells per cubic milliliter as compared with those who delayed or did not initiate HAART (22). Early commencement of HAART for HIV infected patients has also been shown to significantly reduce HIV transmission between sero-discordant couples (22). Knowledge of one`s HIV status is a key determinant for the initiation of HAART. The infrequent HIV testing rates observed in this study among medical laboratory scientists, a high risk group for HIV infection in Africa, may prevent those infected with HIV the critical opportunity for early initiation of HAART. Such people unaware of their HIV positive status may also pose a serious public health hazard and serve as a source of potential transmission of HIV.

In our study an association was observed to exist between HIV status testing in the previous 12 months and the gender of medical laboratory scientists. Female participants were 1 to 7 times significantly more likely to have been tested for HIV in the last 12 months than their male counterparts. Data from the demographic and health surveys on prior HIV status testing suggest a generally higher testing rate among females in Nigeria (23) and other West African countries, (24-27). HIV testing in the previous 12 months was observed to be significantly affected by post qualification experience. Participants with three or less years of working experience were significantly ($P = 0.020$) more likely to have been tested for HIV in the previous 12 months. Participants with higher professional experience are more likely to be senior colleagues at work place who may have over the years, risen to supervisory positions and consequently engage less often in direct processing of clinical samples. They may therefore perceive themselves of having less risk of exposure to infectious pathogens. Area of specialization, affiliation, location of work place and history of blood donation did not significantly affect HIV status testing in previous 12 months among study participants.

HBV and HCV infections are a global health problem. The spectrum of HBV and HCV infections are varied; ranging from inactive carrier states, to chronic HBV and HCV infections, which have the capacity to progress to cirrhosis and hepatocellular carcinoma (28-31). In our study only 41 (37.3%) of participants reported to have had at least one post-qualification HBV screening test. This is lower than the 54.1% previously documented among medical laboratory technicians in another African study (20). Testing for HBV status in the last twelve months was reported by only 12 medical laboratory scientists, representing 10.9% of all study participants. A poor policy of HBV vaccination has also been reported among private and public diagnostic laboratories in Nigeria (13). Other Nigerian studies have reported low rates of hepatitis B vaccination among HCWs (12,32). These studies have been supported with the findings of this study leaving the medical laboratory scientist at risk of workplace HBV infection which, if unresolved over time, could progress to more fatal health consequences.

Post–qualification testing for HCV among study participants was recorded by 17 (15.5%) which is lower than 40.5% recorded among laboratory technicians in a previous African study (20). Only 8 (7.2%) participants reported to have been screened for HCV status in the last 12 months. A previous study indicated that HCV is under-diagnosed and under-reported in sub-Saharan Africa (33). The progression of HCV infection to cirrhosis is often clinically silent, with some patients unaware of their HCV status, until they present with the complications of end-stage liver disease or hepatocarcinoma (32). Early detection of acute HCV infection is important and should prompt consideration of anti-HCV therapy to prevent chronic HCV infection (18). There is presently no vaccine for prevention of HCV infection, making early detection of HCV infection an important aspect of HCV control measures. The poor rate of HCV status testing observed in our study may lead to undetected HCV infection among this class of HCWs, which in turn could progress to chronic HCV infection with its increased risk of morbidity. Medical laboratory scientists who had donated blood were 1 to 24 times significantly more likely to have undergone HCV testing in the previous twelve months than those with no history of blood donation. HCV screening of blood donors is a pre-requisite for safe blood transfusion services worldwide. This probably explains this observation in our study.

It is important to note that that conclusion drawn in our study were based on entries made by medical laboratory scientists in questionnaires, which indeed may have been prone to biased reporting. Participants of this study were from various health institutions in Edo State, Nigeria. The findings therefore may not be representative of all medical laboratory scientists in Nigeria.

The uptake of HBV and HCV testing services by our study participants was overall poor. Although all medical laboratory scientists recruited in this study reported to have had at least one post-qualification HIV status screening test, testing rate 12 months prior to the present study was poor and significantly associated with gender and post-qualification experience of study participants. Participants who had donated blood were significantly more likely to have had an HCV screening test in the previous twelve months. Early detection of disease is critical for effective management and care of patients. Regular status screening tests for HIV, HBV and HCV are advocated for medical laboratory scientists in Nigeria.

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Appendix

QUESTIONNAIRE

This questionnaire seeks to determine the rate and factors associated with uptake of Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) testing services among medical laboratory scientists working in medical diagnostic laboratories in Nigeria. Please your candid opinion on questions asked is highly solicited. Please tick and write where appropriate. Participants and affiliation confidentiality will be strictly maintained.

1. Gender	☐ Male	☐ Female
2. Years of qualification as a medical laboratory scientist	_____	
3. How long have you been practicing as a medical laboratory scientist?	_____	
4. Area of specialization:	_____	
5. What type of laboratory do you work in?	☐ Private laboratory	☐ Public laboratory
6. Where is this laboratory situated?	☐ Urban setting	☐ Rural setting
7. Ever donated blood?	☐ Yes	☐ No
8. Have you been tested for HIV since qualifying as a medical laboratory scientist?	☐ Yes	☐ No
9. Have you been tested for HIV in the last twelve months?	☐ Yes	☐ No
10. Have you been tested for HBV since qualifying as a medical laboratory scientist?	☐ Yes	☐ No
11. Have you been tested for HBV in the last twelve months?	☐ Yes	☐ No
12. Have you been tested for HCV since qualifying as a medical laboratory scientist?	☐ Yes	☐ No
13. Have you been tested for HCV in the last twelve months?	☐ Yes	☐ No

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Fine needle aspiration cytology of chromophobe renal cell carcinoma. A report of two cases

Sarla Naran, Sharda Lallu and Peter Bethwaite

Abstract

We describe the cytologic features of chromophobe renal cell carcinoma (ChRCC) in two cases, the first being a fine needle aspiration (FNA) from a kidney tumour in a 50 year old female and the second an FNA of a soft tissue mass over the anterior chest wall in a 75 year female with a known past history of ChRCC of the left kidney. The features were similar in both cases and revealed cellular samples composed of many dissociated and small groups of polygonal cells with granular to flocculent cytoplasm, well defined cell borders, small nucleoli and distinct perinuclear clearing. Retrospective histochemical and immunohistochemical studies had been performed to confirm the renal origin and chromophobe phenotype. The tumour cells were positive for cytokeratin AE1/AE3, CK7, EMA and CD10 and negative for vimentin and S100. The tumour cells were negative for TTF-1 and thyroglobulin in second case. Histochemical study showed tumour cells that were positive for Hale's colloidal iron stain and negative for PAS and PAS-D. Subsequent fluorescent in situ hybridization (FISH) analysis showed loss of chromosomes 1, 2, 6, 10 and 17. A correct diagnosis of ChRCC may be possible on cytomorphology along with a Hale's colloidal iron stain and confirmed by immunohistochemical and FISH analysis in difficult cases.

Key words: renal cell carcinoma, chromophobe, fine needle aspiration, soft tissue, metastasis, FISH.

N Z J Med Lab Sci 2014; 68: 62-64

Introduction

Chromophobe renal cell carcinoma (ChRCC) is a variant of parenchymal renal cell carcinoma, comprising 2-5 % of all renal cell carcinoma and characterised by unique morphological, histochemical, ultrastructural and genetic features (1-5). ChRCC is biologically less aggressive than clear cell renal carcinoma (3,6,7) having a low potential for metastasis mostly to lymph nodes and liver (3). ChRCC can be confused with clear cell renal cell carcinoma or oncocytoma and several non-renal tumours with overlapping features including hepatocellular carcinoma, adrenal cortical carcinoma, lung carcinoma, and oncocytic tumours of salivary gland and thyroid.

We describe the cytologic features of ChRCC diagnosed by FNA in two cases with histochemical, immunohistochemical and cytogenetic findings.

Case reports

Case 1

A 50 year old female was found to have an incidental left renal tumour on investigation for biliary colic. She had no any urinary symptoms or haematuria. An ultrasound scan showed gall stones and a well defined, round 40 x 30 mm solid left renal tumour. FNA of the left kidney mass was performed under ultrasound guidance. After confirmation of a renal cell carcinoma in the left kidney by FNA, laparoscopic left nephrectomy with transvaginal removal of the tumour was performed.

Case 2

A 75 year old female with history of a renal cell carcinoma of the left kidney who now presented with right shoulder pain and a palpable 80 mm soft tissue mass over the anterior chest wall, deep to the skin, three years after nephrectomy. At that time histology of the left kidney revealed a 165 mm chromophobe renal cell carcinoma which did not breach the renal capsule but did demonstrate vascular space invasion and renal sinus invasion. Ultrasound guided FNA of the right anterior chest wall mass was performed. Core biopsy was not performed as the aspirated material was adequate to make a diagnosis.

Materials and methods

On site smears were made from the FNA material in both cases and fixed in 95% ethanol and stained with modified Papanicolaou method and the rest of the material was collected in 30% ethanol in physiologic saline. From half of this material, filter preparations were made on size 5 micron Sartorius AG-cellulose acetate filters using the cytosieve method and stained by the Papanicolaou method. The remainder of the aspirate sample was spun and from the sediment a cell block was made, fixed in 10% formalin, embedded in paraffin, routinely processed and stained with hematoxylin-eosin (H & E).

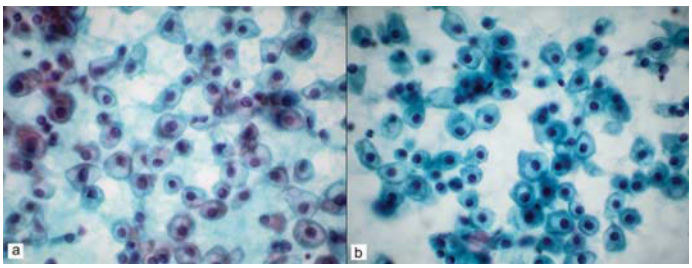
Immunohistochemical studies were undertaken using antibodies CK7 (1:750 Dako), cytokeratin AE1/AE3 (1:1000 Dako), EMA (1:750 Dako), CD10 (1:300 Novocastra), vimentin (1:4000 Dako), S100 (1:4000 Dako), TTF-1 (1:250 Novocastra) and thyroglobulin (1:500 Dako). Electron microscopy (EM) on cell block sections were unsuccessful but were performed subsequently on sections from primary tumour. FISH analysis on cell block sections also were unsuccessful due to the loss of material but were possible on formalin fixed tissue sections of the primary tumour in the kidneys of both cases.

Results

Cytological findings

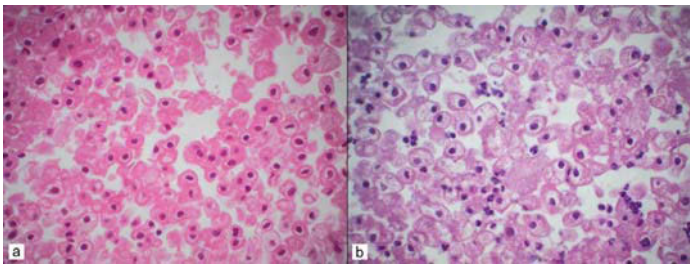
Case 1

Smears and filter preparations were cellular and composed of predominant dissociated and occasional clusters of variably sized neoplastic cells, some with uniform granular cytoplasm and some with flocculent cytoplasm having well defined cell borders, round to oval nuclei without nucleoli and irregular nuclear membrane giving a "raisinoid" appearance (Figure 1a). Rare intranuclear inclusions and grooves were noted. Occasional cells with more pleomorphic nuclei were seen. Perinuclear clearing was evident in cell block section (Figure 2a).



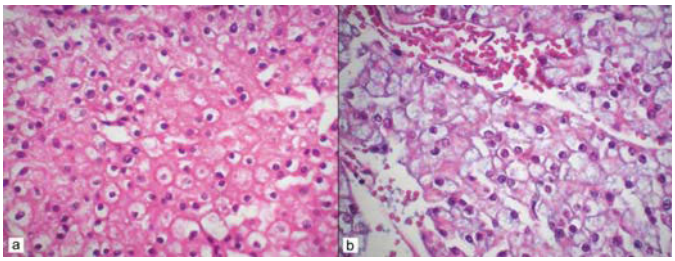
Figures 1a and 1b. Smear preparations from FNA of case 1 (Figure 1a) and case 2 (Figure 1b) showing round to polygonal tumour cells with granular to flocculent cytoplasm, well defined cell borders, and perinuclear clearing. In addition, irregular nuclear membrane (raisinoid appearance) in case 1 (Figure 1a). Papanicolaou stain x 400.

Case 2
Smears and filter preparations were cellular, comprising predominantly single cells and small groups of cells in a background of blood. The cells were large, polygonal to round with granular to flocculent cytoplasm and well defined cell borders. The nuclei were vesicular with small or indistinct nucleoli. Perinuclear clearing was a striking finding in most of the cells on smears and cell block (Figures 1b and 2b). Mild nuclear pleomorphism was present..Large bizarre cells, intranuclear pseudoinclusions, marked irregularity of nuclear membranes (raisinoid features) and nuclear grooves were not evident in this case. Also evident were occasional large papillary fragments lined by large cells with abundant clear to vacuolated cytoplasm.



Figures 2a and 2b. Cell block preparations from FNA of case 1 (Figure 2a) and case 2 (Figure 2b) showing round to polygonal tumour cells with granular to flocculent cytoplasm, well defined cell borders, and striking perinuclear clearing. Hematoxylin-eosin stain x 400.

Histological findings
Case 1
The tumour was composed of trabeculae and sheets of neoplastic cells of varying sizes with flocculent cytoplasm and well defined cell borders separated by thin fibrovascular septa. “Raisinoid” nuclei were evident with predominant nuclear grooves, rare intranuclear inclusions and the presence of a perinuclear clear zone (Figure 3a). In addition, throughout the tumour were scattered larger cells with pale cytoplasm. No extra renal extension and vascular invasion was seen.

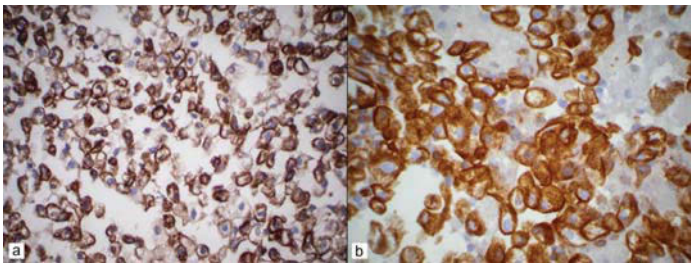


Figures 3a and 3b. Kidney biopsy sections of case 1 (Figure 3a) and case 2 (Figure 3b) showing sheets of neoplastic cells of varying sizes with flocculent cytoplasm, well defined cell borders, and most evident perinuclear clear zone separated by thin fibrovascular septa. Hematoxylin-eosin stain x 400.

Case 2
The primary tumour in the kidney was composed of large sheets of neoplastic cells of varying sizes with pale to flocculent cytoplasm, well defined cell borders and peri nuclear clear zone separated by thin fibrovascular septa (Figure 3b). Some of the tumour cells had irregular nuclear membrane and inconspicuous nucleoli. Focally papillary fragments of tissue lined by large cells with abundant clear cytoplasm (balloon cells) were noted. Vascular invasion was noted.

Histochemical findings
PAS and PAS-D stains on cell block sections were negative and Hale’s colloidal iron stain on sections of cell blocks and primary tumour in both cases were positive showing bright blue cytoplasmic staining in contrast to red nuclei.

Immunohistochemical findings
Immunohistochemical stains on cell block sections in both cases were positive for CK7 (Figures 4a and 4b), EMA and cytokeratin AE1/AE3. CD10 was focally positive. Vimentin and S100 were negative. TTF-1 and thyroglobulin were negative in the second case. Immunohistochemical studies were not done on the resected primary kidney tumour of these cases as morphologic features were classical of chromophobe renal cell carcinoma.



Figures 4a and 4b. Immunohistochemical stain on cell block sections of case 1 (Figure 4a) and case 2 (Figure 4b) showing diffuse positivity for CK7 (CK7 x 400).

EM findings:
Electron microscopy on tissue sections showed focal intra-cytoplasmic microvesicles.

Cytogenetic analysis
FISH analysis on tissue from the nephrectomy specimens showed losses of chromosomes 1 (81%), 2 (74%), 6 (52%), 10 (81%), and 17 (74%) in the first case, and chromosomes 2 (86%), 6 (76%), 10 (98%), and 17 (86%) in the second case.

Discussion

ChRCC was first described by Thoenes *et al*. in 1985 (4). The tumour is characterised by large cells with abundant granular to fluffy cytoplasm with well-defined cell borders and perinuclear clearing (1-9) leading to confusion with clear cell carcinoma and oncocytoma. In difficult cases a positive Hale’s colloidal iron stain (1-7, 9-12) and ultrastructural demonstration of numerous oval cytoplasmic microvesicles establishes the correct diagnosis of ChRCC. The characteristic cytomorphologic features noted correspond to the histologic features and include large polygonal to round cells with granular to flocculent cytoplasm, well defined cell borders, mild nuclear pleomorphism, vesicular nuclei, perinuclear clearing, and small to indistinct nucleoli. ChRCC has been shown to be immunoreactive for CK7 (1,2,5-8), cytokeratin AE1/AE3, EMA, and to be negative for vimentin (1,2,5,7 8) as seen in these two cases with positive Hale’s colloidal iron stain and negative PAS and PAS-D stain. Ultrastructural study has demonstrated oval cytoplasmic microvesicles (1-4,7,9-11) which were focally seen on primary tumours in both cases. ChRCC shows loss of chromosomes 1, 2, 6, 10 ,13, 17 and 21 which may be used to confirm the diagnosis of ChRCC (10-12).

Despite the excellent prognosis associated with ChRCC, metastases may rarely develop, especially in large multifocal tumours. The presence of sarcomatoid elements, tumour size > 80 mm, tumour necrosis, and vascular invasion have been associated with poor prognosis (5,8). Invasion was present in the primary tumour of the second case. Although the cytologic features of ChRCC are unique, several tumours, such as renal oncocytoma, clear cell carcinoma, granular cell carcinoma, hepatocellular carcinoma, adrenal cortical carcinoma, lung carcinoma, and oncocytic tumours of salivary gland and thyroid must be considered in the differential diagnosis.

The presence of variegated cytoplasm and frequent perinuclear clearing in ChRCC and presence of uniform large cells with abundant homogeneous granular cytoplasm, lacking perinuclear clearing, and nuclear membrane irregularities, helps in distinction from renal oncocytoma (1,2,6,7,9,11,12).

In the current cases, characteristic morphologic features of ChRCC, diffuse strong positive CK7 ruled out the diagnosis of a renal oncocytoma. The absence of cytologic features of clear renal cell carcinoma, such as large clusters of cells with abundant clear to finely vacuolated or granular cytoplasm, many stripped nuclei, centrally located nuclei, prominent nucleoli, more uniform nuclear size, lacking perinuclear clear zone, and the positive CK7 in these cases and negative vimentin, excluded clear cell carcinoma. Hale's colloidal iron stain is negative in clear cell carcinoma. The distinction from granular cell variants of renal cell carcinoma may be made by the presence in the later of large cells with abundant granular cytoplasm, ill-defined cell borders, prominent nucleoli, and intermixed variable number of clear cells, negative Hale's colloidal iron stain, cytokeratin, EMA, and positive reactions for vimentin, S100 and CD68.

Metastatic ChRCC appears most commonly in the liver and lymph node, rarely at other sites, and must be distinguished from tumours of non-renal origin with superficial resembling features such as hepatocellular carcinoma, adrenal cortical carcinoma, lung carcinoma, and oncocytic tumours of salivary glands and thyroid. These possibilities were excluded as imaging of liver, pancreas, spleen, adrenals and lung was normal in both cases. TTF-1 and thyroglobulin were negative in the second case making origin from lung or thyroid unlikely.

Conclusions

Although the cytologic features of ChRCC have been previously described, there have been only a few reported cases of ChRCC diagnosed on FNA material (1-3,7,9) and very few metastatic examples diagnosed using FNA (1-3,9). A correct diagnosis of ChRCC may be possible on cytomorphology along with a Hale's colloidal iron stain and confirmed by immunohistochemical and FISH analysis in difficult cases.

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A simple functional assay for heparin-induced thrombocytopenia/thrombosis syndrome using a full blood count analyser

George TC Chan, Sophie HS Lee and Simon DF Jones

Abstract

Objective: Heparin-induced thrombocytopenia (HIT) is a complication of heparin use due to development of HIT antibody which activates platelets resulting in a prothrombotic state. Early diagnosis and management is essential to prevent critical ischaemia or even death resulting from uncontrolled thrombosis. A rapid, sensitive and specific functional laboratory diagnostic test, however, is not available in most hospitals. We aimed to develop a simple assay based on the drop in platelet count of normal donor platelet-rich plasma in the presence of HIT antibody and heparin.

Materials and methods: Stored HIT positive and negative plasmas were mixed with normal platelet-rich plasma, heparin at different concentrations, or saline. The platelet counts of the mixtures before and after incubation were determined on a routine blood cell counter.

Results: The platelet count dropped significantly in the presence of HIT positive plasma and therapeutic concentration of heparin due to platelet aggregation. The platelet counts dropped only slightly in the control tubes, which could be explained by dilution and non-specific clumping.

Conclusions: This proof-of-principle finding suggests this simple test, which can be done in any haematology laboratory with a modern blood cell counter, holds great promise as a simple functional test for laboratory diagnosis of HIT. Further work is required to validate the results.

Key words: heparin-induced thrombocytopenia, functional assay, platelets

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Introduction

Heparin-induced thrombocytopenia (HIT) is a serious complication from heparin use, arising from the binding of antibody against heparin-platelet factor 4 (PF4) complexes onto platelets. This activates the platelets, resulting in a prothrombotic state with the risk of arterial or venous thrombosis, as well as thrombocytopenia from the platelet consumption (1).

The Heparin-PF4 antibodies (HIT antibodies), which are mainly of IgG class, usually develops 5 to 10 days in susceptible patients after heparin exposure. The incidence of HIT is between 0.5-5% of patients treated with heparin (2). It is higher in surgical patients than medical patients, and with unfractionated heparin compared to low molecular weight heparin. If untreated, 30-50% of patients with HIT develop severe thrombotic problems including skin necrosis, venous gangrene, limb complication, or even death. The treatment is to stop the heparin and use alternative anticoagulants such as direct thrombin inhibitors or direct-Xa inhibitors (3,4).

Danaparoid was used previously with success, but this product is no longer available. While the newer anticoagulants can be effective to stop the haemostatic activation, experience in their use is limited. Other disadvantages include difficulty monitoring the therapeutic effect, lack of effective antidotes for anticoagulant reversal in case of bleeding from overdose, and higher cost. Early diagnosis and management of HIT is therefore essential to prevent the morbidity or even death resulting from the uncontrolled thrombosis, and to avoid the unnecessary use of alternative anticoagulants.

The HIT diagnosis is based on clinical and laboratory criteria (5). The laboratory testing of HIT falls into immunological or functional groups (6). The immunological tests include enzyme-linked immunosorbant assays (ELISA) and particle-gel immunoassays (PaGIA). They are used to detect the presence of HIT antibodies. They are sensitive and can provide the results relatively rapidly. ELISA typically can produce the results in two to four hours, whereas the PaGIA has a turn-around time of within one hour. Both tests, however, have low specificity for the diagnosis of HIT, as they cannot distinguish between heparin-PF4 antibodies that are non-platelet-activating and hence innocuous, or pathological platelet-activating antibodies. The percentage of patients on heparin who develop HIT antibody is much higher than the actual incidence of clinical HIT. This difference is seen in all patient groups, but the cause is not known (2). A classic example is after cardiopulmonary bypass operations in which up to 50% of patients develop HIT antibodies by the fifth post-operative day, but clinical HIT is seen in 2-3% of post-cardiopulmonary patients only.

The laboratory diagnosis therefore frequently requires functional assays to demonstrate the platelet activation by the HIT antibody. The current functional tests include serotonin release assay, heparin-induced platelet aggregation test using platelet-rich plasma (PRP), and the emerging whole-blood impedence platelet aggregometry (7). They are specific but time consuming and require specialised skills and equipment. They are therefore usually done in specialised/referral centres only, and the results are not available for immediate patient management.

We aimed to develop a simple functional assay which can be done in all clinical laboratories with a modern blood cell counter with the result available rapidly, to facilitate the rapid confirmation of HIT for optimal patient management. The assay we have devised is based on the pathological mechanism of HIT: that the true pathogenic HIT antibody will activate platelets. One of the consequences would be platelet clumping, leading to a drop in the platelet count. We hypothesise that this drop in the platelet count in a donor blood specimen after exposure to heparin and the plasma from a patient suspected of HIT can be used to demonstrate the presence of the pathological HIT antibody.

Methods

Stored plasmas from patients with suspected HIT were used to develop the test. These plasmas were anonymised after the original clinical tests and stored as controls for HIT testing; therefore only a limited number of these samples were available. The HIT positive plasmas were confirmed by heparin-induced-platelet aggregation test (HIPAT) using light aggregometry and normal platelet-rich plasma. The negative plasmas were negative with a Particle-Gel-Immunoassay (ID-PF4/heparin antibody test (Diamed AG, Cressier, Switzerland).

Citrated blood was collected from volunteer laboratory staff not on medication affecting platelet function and whose platelets had previously shown to be sensitive to platelet-activating HIT antibodies. Normal platelet-rich plasma (NPRP) was prepared by centrifuging at 170g for 10 minutes at 20°C and rested for 30 minutes at room temperature prior to testing.

After the initial optimisation, the test protocol was established as follows:

- Platelet count was performed on normal donor PRP using a Sysmex XE5000™ (Sysmex, Kobe, Japan).
- Four plastic centrifuge tubes were labelled and the following added:

  Tube 1: 200 uL NPRP + 100 uL test plasma + 20 ul 8 IU/mL heparin.
  Tube 2: 200 uL NPRP + 100 uL HIT negative (normal donor) plasma + 20 uL 8 IU/mL heparin
  Tube 3: 200 uL NPRP + 100 uL test plasma + 20 uL 1,600 IU/mL heparin.
  Tube 4: 200 uL NPRP + 100 uL test plasma + 20 uL saline.
- The tubes were inverted 5 times to gently mix the contents, incubated in a 37°C water-bath for 15 minutes, with gentle mixing every 5 minutes.
- After incubation, the platelet counts in the tubes were determined, and the results compared to the original platelet count of the NPRP.

Tube 1 was the test tube, Tube 2 the main control tube and Tubes 3 and 4 were additional control tubes. The test was done on the only remaining HIT positive plasma, six HIT negative plasma and the HIT-positive control provided in the PaGIA kit by the manufacturer.

Smears from Tube 1 of the HIT positive specimen before and after incubation were also made and stained with May-Grunwald-Giemsa stain to check for platelet aggregates.

Results

HIT-positive patient specimen

The platelet counts of the normal PRP and the mixture in the tubes after incubation for the HIT-positive case are summarised in Table 1 and Figure 1. The only sample that showed a significant drop in platelet count was the HIT positive plasma with 8 IU/mL heparin. The platelet count dropped from 384 x 10^9/L pre-incubation to 40 x 10^9/L post-incubation, with a post-test/normal PRP platelet count ratio of 0.10. The May-Grunwald -Giemsa stained smears confirmed that the drop in the platelet count was due to platelet aggregation (Figure 2).

Table 1. Platelet counts and post-test/normal PRP platelet count ratio

	Normal PRP	Post test			
		Tube 1	Tube 2	Tube 3	Tube 4
Platelet count (x10^9/L)	384	40	266	234	240
Ratio of Post-test /Normal-PRP platelets	--	0.10	0.69	0.61	0.63
Ratio of platelet count compared to the control (Tube 2)	--	0.15	1	0.89	0.90

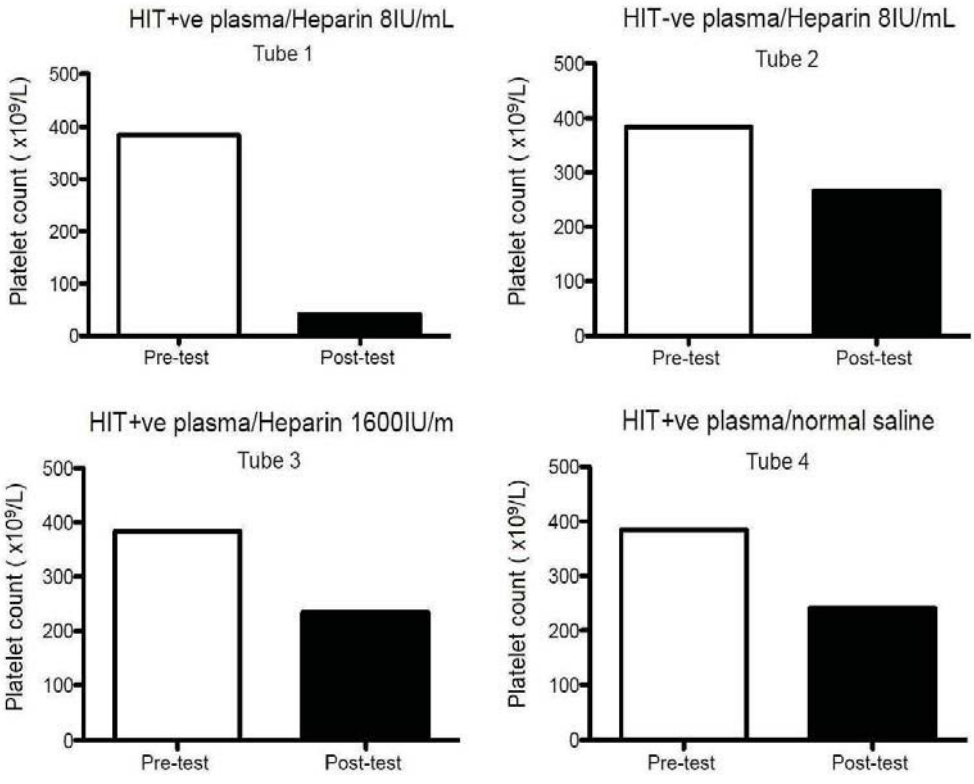

Figure 1. Platelet count changes after incubation

The platelet counts in the control tubes (Tubes 2 to 4) also dropped after incubation but this could be explained by dilution and the mild platelet clumping due to the charge effect from the heparin (8). The post-test/normal PRP platelet count ratios in the control tubes were much higher (range 0.61-0.69).

The results can also be expressed by the platelet count ratio in the tubes compared to the main control (Tube 2 in the test protocol which contains HIT-negative, normal donor plasma with therapeutic concentration of heparin). This ratio is 0.15 for the HIT-positive plasma and around 1.0 for the other control tubes (Table 1).

HIT-negative patient specimen and commercial positive control

The range of the platelet count ratio of test/control tube for the HIT-negative specimens were 0.91 to 1.06, and for the commercial HIT-positive control was 0.08 (Table 2).

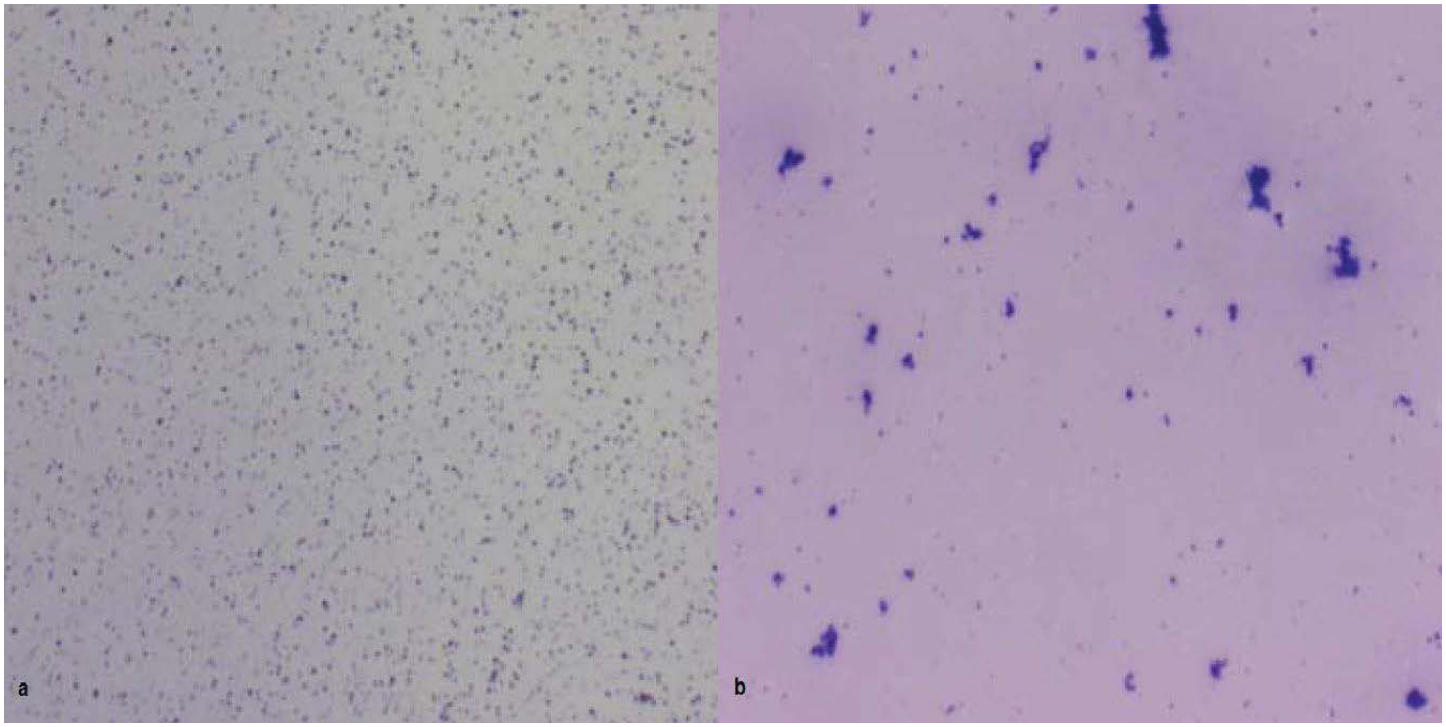

Figure 2. Smears made from Tube 1 containing NPRP, HIT+ plasma and 8 IU/mL heparin, stained by May-Grunwald-Giemsa stain and examined at 100X magnification. (a) Before incubation showing dispersed platelets. (b) After incubation showing large platelet clumps.

Table 2. Platelet count ratio of HIT-positive and HIT-negative specimens

	HIT-positive specimen	HIT-positive commercial control	HIT-negative specimens (n = 6)
Platelet count ratio of Test/ Control tubes	0.15	0.08	0.91 – 1.06

Discussion

This simple assay using a modern blood cell counter and based on the pathological mechanism of HIT syndrome shows great promise as a functional test for the laboratory diagnosis of HIT. The large drop in platelet counts in the HIT-positive patient specimen and commercial control but not in the HIT-negative specimens suggests this observation is genuine and not a spurious, chance finding.

Because of the constraint of the availability of HIT positive and negative specimens, however, the present results are only a proof-of-principle finding. As only one HIT positive specimen was available, the cut-off of the test/control platelet ratio in order to call a result positive cannot be determined from this study.

Further work is required to refine the test procedure, to validate the result on a large number of HIT positive and negative specimens, to understand its performance characteristics including the sensitivity, specificity and predictive values for HIT, and to define its role in conjunction with the rapid antigenic test for HIT diagnosis.

We also tried using EDTA whole blood as the source of platelets and incubating blood group 'O' donor blood with the test mixtures at room temperature. If successful this could expand the pool of donor platelets instead of relying on voluntary donors, and obviate the need to prepare platelet rich plasma. Our initial experiment however resulted in spontaneous non-specific platelet aggregation. Further optimisation could not be achieved due to difficulty obtaining more HIT positive plasma.

In conclusion, this simple assay using a routine blood cell analyser shows great promise as a new functional laboratory test for HIT testing. Because of its simplicity, even with platelet-rich plasma as the indicator system, the test can be done in all contemporary haematology laboratories, and the result is available rapidly. It can be particularly useful in cases where the specialised functional tests are not readily available. Even if further validation studies show this test does not reach the sensitivity and specificity to be used as a stand-alone diagnostic test, provided that it still has reasonably high positive and negative predictive values, it may still be useful clinically in conjunction with a rapid immunoassay such as PaGIA for rapid exclusion or confirmation of HIT in many patients, and reduce the need for the complex diagnostic functional tests.

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News from the Universities

Interview with AUT's BMLSc 2013 NZIMLS prize-winner Katja Campbell

Katja Campbell is the top academic graduate of Bachelor of Medical Laboratory Science from AUT in 2013. As such, Katja wins the NZIMLS prize and is grateful for this financial support from NZIMLS. Katja specialised in Microbiology and Immunology and also topped the class in her two specialisations. Holly Perry from AUT interviewed Katja about her academic success and love of medical laboratory science.

Holly: What made you decide to become a medical laboratory scientist?
Katja: I have wanted to work in a health field since I was quite young. I loved Science at School, and Science and Health are a good combination for medical laboratory scientists. In Finland, I qualified as a medical laboratory scientist in 1994. When I moved to New Zealand, I wanted to get my scientist registration in New Zealand, and so I came to AUT to study the NZ BMLSc degree.

Holly: What do you like about medical laboratory science in general, and Microbiology and Immunology in particular?
Katja: I like the variety of the work; in Microbiology there is a puzzle for scientists to solve; what might be causing the patient's disease. I like bacteriology, parasitology and mycology, but probably like bacteriology the best. In Immunology, there are also specialist areas within the discipline, so the variety available to medical laboratory scientists is really good.

Holly: You had your clinical placements in Finland. How did you find that experience?
Katja: Really good. I had worked in Helsinki University hospital previously, so knew what to expect. I got to catch up with some old school friends who are now working in the lab. The technology levels there and in New Zealand labs are very similar; for example we had MALDI-TOF there, and I know that is in many New Zealand labs now. In Immunology in Finland, there was a mix of manual serology and automated assays; similar to here. In Virology, a lot of molecular methods were used.

Holly: What study skills do you recommend to other BMLSc students to achieve success?
Katja: Always strive to do your personal best, to the best of your ability. Put time and effort into your studies, and prioritise your study.

Holly: Where are you working?
Katja: I am enjoying work at New Zealand Blood Service in Auckland

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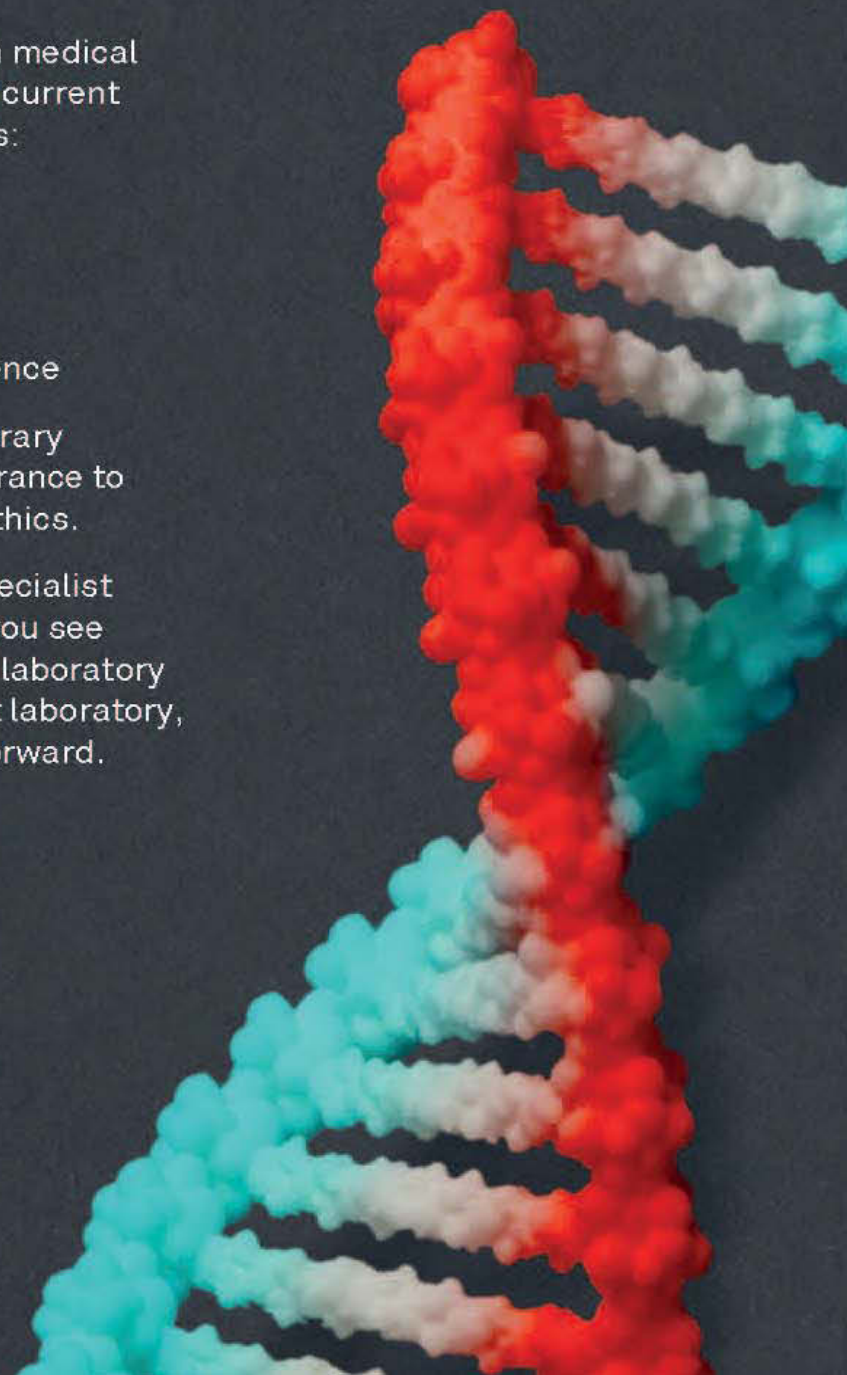


NZIMLS Histology SIG
Tauranga 2014

House of Wax

Come and join us for a day in the beautiful Bay of Plenty. Be part of some great histopathology presentations. Then dress up for dinner as your favourite figure who has been immortilised in wax. (Dress up encouraged, but optional)

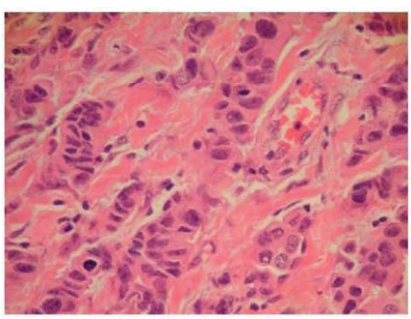
Think Madame Tussauds, House of Horrors, Celebrities, Sport Stars and Superheroes.

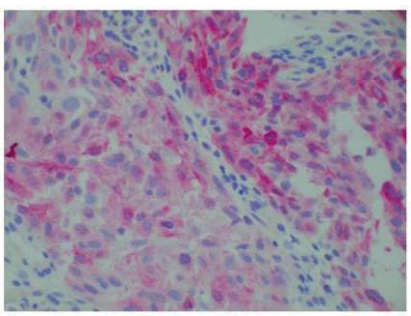
Saturday 11th October
Classic Flyers,
9 Jean Batten Drive
Mount Maunganui

Submit presentation proposals to
Corinne Hill
corinne.hill@pathlab.co.nz

More details to follow www.nzimls.org.nz

Who do you want to be?





Haematology SIG

Wellington 18th October 2014

To be held at Macs Brewery Function Centre, Taranaki Street Wharf, Wellington.

Presentations are invited for all aspects of Haematology

Get your registrations in quick!

Contact: Raylene Allan-Sloper

RAllan-Sloper@apath.co.nz

PRE-ANALYTICAL SERVICES

Special Interest Group

NZIMLS

Annual Seminar

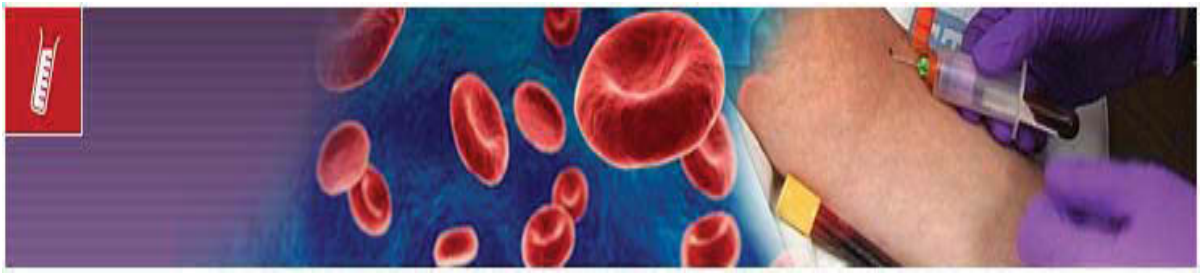
SATURDAY 18 OCTOBER

WAIPUNA HOTEL

Mt Wellington, Auckland. www.waipunahotel.co.nz

- REGISTRATION + COFFEE 9:30am
- SESSIONS START AT 10:00am
- FINISH AT 16:3Opm

Phlebotomy & Specimen Services



CONTACT: NZIMLS PAS SIG CONVENORS:
Specimen Services: Joanna Barnes: joanna.barnes@waitematadhb.govt.nz
Phlebotomy: Annette Bissett: annette.bissett@waitematadhb.govt.nz
Online registrations available http://www.nzimls.org.nz/

Immunology

Special Interest Group - ISIG

Saturday 8th November 2014

ibis Hamilton Tainui Hotel

from 9.00am

Welcome to the Waikato!

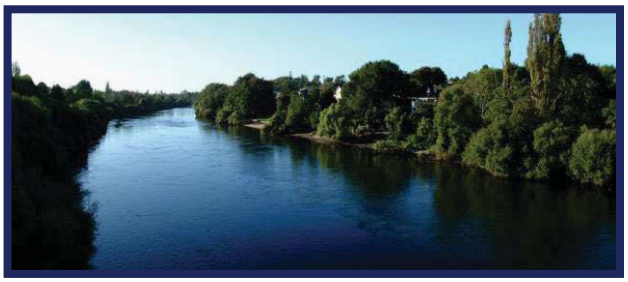
Land of Milk and Honey, Thoroughbreds and Sports Stars

Venue: IBIS Hotel, Alma Street, Hamilton,

ph.07 838 1366

Presentations are invited from all aspects of Immunology and Virology
For presentations please contact Tim Taylor at tim.taylor@pathlab.co.nz

Several accommodation choices are available within easy walking of this central Hotel. Special rates are also available at the IBIS.



Please join us at the IBIS at end of the ISIG to catch-up with friends and colleagues, with drinks and nibbles, overlooking the mighty Waikato River.

Further information and registration details will soon be available at
www.nzimls.org.nz

Mortuary SIG Seminar
29 November 2014
Rotorua

Novotel Rotorua Lakeside

Presentations from:
Rotorua Coroner
New Zealand Police
Dr M Arendse, Pathologist
Dr Nadir Hasan, Pathologist
St Johns Ambulance Service
Trainee Mortuary Technician Rotorua
Funeral Director FDANZ
General Manager Maori Health Lakes DHB
Merelyn Redstone, NIIO

Any enquiries: jason.sayers@lakesdhb.govt.nz

Register on-line **www.nzimls.org.nz**

Laboratory quality management in the Pacific Islands

The PPTC has established its activity calendar for 2014 and continues to deliver guidance and education in laboratory quality management to Vanuatu, Cook Islands, Kiribati, Tonga, and Samoa. The programme has been further extended for 2014 (through the generous sponsorship of The New Zealand Ministry of Foreign Affairs and Trade) and will incorporate the Marshalls, Niue, Nauru, Christmas Island, and Tuvalu.

In March of this year, Phil visited Kiribati while Russell made a visit to Tuvalu and Navin to the Marshall Islands. Following on in April, Russell visited Nauru, and in May Navin visited both Christmas Island and Vanuatu. It was very fortunate to have Koen Van der Werff, Section Head, Microbiology, Wellington Hospital, as a consultant for Niue in May and at the same time Phil visited Tonga to carry out LQMS implementation as well as Haematology teaching.

In June of this year, Navin revisited Tuvalu to assess the progress made in LQMS since Russell's visit and Russell revisited the Marshall Islands to assess progress made since Navin's visit. Phil is scheduled to travel to the Philippines to meet with representatives associated with WHO in Manila and then onto Palau to carry out an REQA assessment of the laboratory. The remainder of 2014 with no exception is packed with activity with repeated visits scheduled for Tonga, Kiribati ,Samoa, Cooks, and Vanuatu. American Samoa, Pohnpei, Chuuk, Yap and Kosrae will also be visited this year.

Pacific Paramedical Training Centre updated training courses 2014

Haematology and blood cell morphology	4 – 29 August 2014
Microbiology	1 – 26 September 2014
Laboratory quality management systems	29 – 24 October 2014
Blood transfusion science	3 – 28 November 2014

For further information contact:

Navin Karan, Programme Coordinator
PPTC, PO Box 7013 Wellington, New Zealand
Telephone: +64 4 389-6294; Fax: +64 4 389-6295,
Email: pptc@pptc.org.nz. Website: www.pptc.org.nz,

Online Distance Learning Courses

The PPTC this year is offering the final three POLHN modules (microbiology, transfusion science, and laboratory quality management) leading to the Diploma in Medical Laboratory Science [PPTC] to students who have met all first year requirements. This current programme cycle began in March 2013 and the scheduled dates for the remaining modules to be released in 2014 are as follows:

2014 Modules:

Microbiology:	released in March 2014
Transfusion Science:	17 June 2014
Laboratory Quality Management Systems:	17 September 2014
Examination Part 2:	1 December 2014

Welcome to our new PPTC Board member

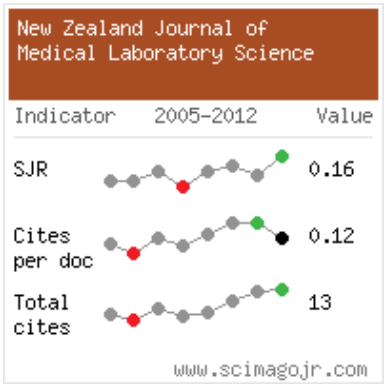
It is of great pleasure that we welcome John Elliott to the PPTC Board of Governance.

John became the Director of the PPTC in 2000 and after completing 11+ years of devoted service, decided it was time to take life a little easier, and so, on the 3rd February 2012. he retired from this position. John made a phenomenal contribution to Pacific Health throughout his time as Director, and the PPTC Board of Governance are privileged that he wishes to continue this contribution as a Board member and information resource.

Contact us:

Postal address
PO Box 7013
Wellington 6242

Phone and E-Mail
Telephone (64) (4) 389 6294
email:pptc@pptc.org.nz



The SJR indicator measures the scientific influence of the average article in the journal. It expresses how central to the global scientific discussion an average article of the journal is.

Cites per doc (2y) measures the scientific impact of an average article published in the journal. It is computed using the same formula as that of the journal Impact Factor ™ (Thomson Reuters).

Journal questionnaire

Below are 10 questions based on articles in the August 2014 Journal issue. Read the articles carefully as most questions require more than one answer.

Answers are to be submitted through the NZIMLS web site. Make sure you supply your correct email address and membership number. It is recommended that you write your answers in a word document and then cut and paste your answers on the web site.

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

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

The site will remain open until Friday 17th October, 2014. You must get a minimum of 8 questions right to obtain 5 CPD points.

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

August 2014 journal questions

1. Extended spectrum β-lactamase-producing organisms have the ability to hydrolyse which β-lactam antibiotics?
2. What was the main conclusion of the study by Lippi and Ippolito?
3. What was the limitation of the study by Lippi and Ippolito, and what do the authors recommend to overcome this limitation?
4. What constitutes the most important route of HIV, and hepatitis B and C virus infection in health care professionals?
5. What has been shown to significantly reduce HIV transmission between sero-discordant couples?
6. In difficult cases of chromophobe renal cell carcinoma (ChRCC) what features establishes the correct diagnosis?
7. What has been associated with poor prognosis of ChRCC?
8. If untreated, patients with heparin-induced thrombocytopenia (HIT) can develop which thrombotic problems?
9. What is the treatment for HIT?
10. What are the current functional laboratory tests to demonstrate platelet activation by the HIT antibody?

Questions and answers for the April 2014 journal questionnaire

1. Which different hypotheses exist for the direct role of HLA-B27 in the pathogenesis of spondyloarthropathies?
   The arthritogenic peptide hypothesis; the misfolding hypothesis; molecular mimicry.
2. What are the advantages of the fluorogenic real time PCR method for the detection of HLA-B27?
   Lesser hands-on time; does not require any post amplification processing; does not use the toxic DNA intercalcalating dye ethidium bromide.
3. Bacterial isolates harbouring NDM-1 are reported to be resistant to which classes of antibiotics?
   Beta-lactams, fluoroquinolones, and aminoglycosides.
4. Thermo-tolerant *Campylobacter* species are a major cause of which infections; and which is the most prevalent species?
   Food- and water-born gastrointestinal infections. *C. jejuni*.
5. What has proven to be one of the main sources of *Campylobacter* infection in New Zealand?
   Poultry meat.
6. Patients with translocation carcinoma usually present symptomatically with which symptoms?
   Hematuria, abdominal pain, abdominal mass, or fever.
7. Definite diagnosis of translocation carcinoma requires what?
   Immunohistochemical and/or cytogenetic/molecular studies.
8. Where should there be a high suspicion for translocation carcinoma?
   In tumours showing papillary and nested patterns, where there is a mixture of clear and eosinophilic granular cells.
9. Melanoma may mimic which variety of epithelial and nonepithelial tumors?
   Poorly differentiated carcinoma, lymphoma, and pleomorphic sarcoma.
10. What are typical cytological features of papillary thyroid carcinoma?
    Longitudinal nuclear grooves and intranuclear cytoplasmic inclusions.

NZIMLS Annual Scientific Meeting 2014
One Focus
WORKSHOP PROGRAMME

Tuesday 12 August 2014	
WS01	**Auditing / ISO15189:2012**
Presenter	Shelli Turner, IANZ
Workshop Description	The objectives of the workshop is to provide participants with practical knowledge of how to carry out an audit against ISO 15189:2012 and to acquire techniques that will assist with completing an effective internal audit within their own laboratories. The workshop will include creating checklists, the auditing process, recording and reporting audit findings, addressing non-conformances identified and completion of the audit process.
WS02	**Tissue Typing**
Presenters	Paul Dunn, Carolyn Humphries and Deborah Griffiths, NZBS Auckland
Workshop Description	The National Tissue Typing Laboratory is based in the Blood Service in Epsom, Auckland and is responsible for tissue typing tests in support of New Zealand's Stem Cell and Solid Organ transplant programs. The Laboratory also carries out testing for disease association markers, for tissue types associated with hypersensitive drug reactions, for antibodies implicated in transfusion-related reactions and is responsible for the provision of compatible or matched platelets. In this Workshop we will explain in detail the processes involved in tissue typing through videos, slide presentation and case studies.
WS03	**Haematology Morphology**
Presenter	Dr Shingi Chiruka, Haematologist, Dunedin Hospital
Workshop Description	Shingi will present a short talk about morphology as a diagnostic tool, then 20 – 30 study cases will be presented. All participants will have about 3 minutes to look at the microscope and write his/her short comments. The third part will be a discussion about our findings in these cases. This workshop will be fantastic opportunity for very one to share their knowledge and experience in morphology. This workshop will be run in the way as RCPA morphology.
WS04	**A Focus on Parasitology**
Presenters	Tom Henderson & Leonie Thorpe, Canterbury Health Laboratories
Workshop Description	1100 – 1230 An overview of Entamoeba species, update on Dientamoeba, interesting case studies 1330 – 1430 Case studies and paper reviews 1500 – 1600 Update on new diagnostics and multiplex PCR trials General discussion of methods, stains and fixatives
WS05	**Scientific Writing and Methodology**
Presenters	1. Rob Siebers, Editor NZIMLS Journal - Scientific Writing 2. Katrina Sharples, University of Otago - Biostatistics and their use 3. Erin Retter, IANZ 4. Cat Ronayne, University of Otago
Workshop Description	1. Scientific Writing - How to prepare articles for publishing in scientific journals. 2. Biostatistics and their use 3. Verification and Validation from An IANZ perspective - The objective of this segment of the Scientific Writing workshop is to explain what information/documentation is required by IANZ when new test methods or equipment are introduced into your laboratory. Addressing terminology such as 'Validation' and 'Verification' which are frequently mentioned as interchangeable terms, but are in fact quite different and the importance of sign-off will be covered. In addition the ISO 5189:2012 requirements for writing up a test method will be discussed. 4. Cat will outline key points for giving a good oral presentation, including tips, common pitfalls and how to get the most out of PowerPoint. She will also briefly show how PowerPoint can be used to create a scientific poster.
WS06	**Introduction to Management for Medical Laboratory Scientists**
Presenter	Ross Hewett, Laboratory Manager, LabPlus, Auckland City Hospital
Workshop Description	This workshop is designed to provide an introductory overview of the core elements of management within a pathology and diagnostic medical laboratory environment. It will be suitable for scientists and technicians entering or in section leader or technical specialist roles. Topics covered will include process flow and the core elements of a pathology laboratory; staff structure, recruiting, training, competencies and appraisals; financials, capex, opex and business cases; communication and meetings; behaviour issues, quality frameworks and professional responsibilities and current legislation. Because of time, the various topics will not be discussed in any detail, but participants will be provided with sufficient information to research further the various elements of management and perhaps enrol in a more formal educational management course.

NZIMLS Annual Scientific Meeting 2014
One Focus
PRELIMINARY SCIENTIFIC PROGRAMME

Wednesday 13 August 2014	
Official Opening	
Opening Address	Terry Taylor, Conference Convenor
NZIMLS Awards	Ken Beechey, President NZIMLS
TH Pullar Address	Dr Alex Dempster, Southern Community Laboratories
Morning Plenary Session	
Session	One Focus: The Patient. Laboratory Science that creates positive outcomes for our patients
NZIMLS AGM	
Afternoon Plenary Sessions	
Session	Intensive Care & Trauma Medicine. The importance of laboratory testing in Emergency Medicine
Session	Early Life Medicine. The importance of laboratory testing in Reproductive and Neonatal Medicine
Session	Metabolic disease & disorders of major organs. The importance of laboratory testing in supporting organ failure & transplantation
Session	Later Life Medicine. The importance of laboratory testing in supporting our elderly patients
Thursday 14 August 2014	
Morning Plenary Sessions	
Session	Health & Well Being. The importance of laboratory testing in supporting healthy lifestyles
Session	Health & Well Being. Biomarkers in Malignancies
Session	Histology
Session	Proffered Papers
Afternoon Plenary Sessions	
Session	Community Health. The importance of laboratory testing in protecting our communities
Session	Patient's Perspective. How do patients view the medical laboratory?
Session	Cytology
Session	Travel Medicine. The importance of laboratory testing in safe travel
Session	Patient's Perspective. The importance of customer service and cultural competence
Friday 15 August 2014	
Morning Plenary Session	
Session	Quality, Risk & Safety. The importance of getting it right
Closing Plenary	
Session	One Focus – Patients' experience of medical testing
SOCIAL PROGRAMME	
Tuesday 6.30pm	Icebreaker / Opening of the Exhibition
Wednesday 5.00pm	Poster Session
Thursday 7.00pm	Conference Dinner – Theme: ***GOLDRUSH***

For a detailed programme please visit: www.nzimls.org.nz

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THE SOUTH PACIFIC CONGRESS

MONDAY **17 AUGUST** TO FRIDAY **21 AUGUST 2015**

THE LANGHAM, AUCKLAND, NEW ZEALAND

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NZIMLS

VIDE ET DILIGENTIA

2014 NZIMLS CALENDAR *Dates maybe subject to change*		
Date	**Seminars**	**Contact**
11 October 2014	Histology SIG Seminar, Tauranga	corinne.hill@pathlab.co.nz
18 October 2014	Haematology SIG Seminar, Macs Brewery, Wellington	rallan-sloper@apath.co.nz
18 October 2014	PreAnalytical Seminar, Waipuna Hotel & Conference Centre, Auckland	annette.bissett@waitematadhb.govt.nz joanna.barnes@waitematadhb.govt.nz
08 November 2014	Immunology SIG Seminar, Hamilton	tim.taylor@pathlab.co.nz
29 November 2014	Mortuary SIG Seminar, Rotorua	r.j.sayers@actrix.co.nz
Date	**NZIMLS Examinations**	**Contact**
05 November 2014	QMLT and QSST Examinations	fran@nzimls.org.nz
11 – 12 November 2014	Fellowship Examinations	fran@nzimls.org.nz
Date	**Council**	**Contact**
10 – 11 August 2014	Council Meeting, Dunedin	fran@nzimls.org.nz
13 August 2014	Annual General Meeting, Dunedin	fran@nzimls.org.nz
November 2014	Council Meeting	fran@nzimls.org.nz
Date	**Events**	**Contact**
12 – 15 August 2014	Annual Conference, The Dunedin Centre, Dunedin	terry.taylor@sclabs.co.nz fran@nzimls.org.nz

2015 NZIMLS CALENDAR *Dates maybe subject to change*		
Date	**Events**	**Contact**
18 – 21 August	NZIMLS / AIMS South Pacific Congress The Langham, Auckland	rossh@adhb.govt.nz

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[2] Not available via the Pharmaceutical Schedule.

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