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Publication of abstracts of the Annual Scientific Meeting of the NZIMLS

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Last year, abstracts from the Rotorua NZIMLS Annual Scientific Meeting (ASM) were published in the November issue of the Journal, as has been the case in preceding years. Many of those abstracts were, in my opinion, of little use to readers of the Journal. An informative abstract will inform the reader why and how the study was done, and what the main results and conclusions were. In an accompanying Editorial I questioned whether it was of use to continue to waste valuable Journal space printing ASM abstracts containing little or useless information (1).

For the recently held ASM in Auckland, potential contributors were provided with brief, but pertinent guidelines on abstract submission. Looking through these abstracts, there has been a definite improvement in abstract quality compared to previous years. This was, in my opinion, also due to the diligence of the Auckland ASM scientific program convenor, Bryan Raill. There were some excellent abstracts submitted. Here are some that stood out, look them up to see what an informative abstract should look like.

- Wheen L, Moody N, McLelland A, Boswell DR. Early prediction of outcome by Troponin I, Troponin T and CKMB in acute chest pain without electrocardiographic evidence of ischaemia or infarction.
- Mikkelson D, Sacks S, Glen D, Aish L. False positive Troponin I due to platelet interference in the AxSYM Troponin I assay.
- Deroles-Main JR. Evaluation of two rapid tests for the detection of methicillin resistance in Staphylococcus aureus.
- Speedy DB, Noakes TD, Boswell T, Thompson JMD, Rehrer N, Boswell DR. IONman or IRONman? Response to a fluid load in athletes with a history of exercise associated hyponatremia.
- Al-Anbuky N. Significance of toxin(s) A and B enzyme immunoas say for detecting C. difficile in stool specimens.

In this issue of the Journal, abstracts of presentations at the Auckland ASM are published. However, some of you (members, non-members, invited and overseas speakers) may ask why your abstract has not been published. This is because your abstract, in the Editor's opinion, did not meet his guidelines of an informative abstract. Many of these rejected abstracts contained the two most commonly abused phrases, "results (cases) will be presented", and "results (cases) will be discussed". Also, in some of the published abstracts, I have taken out the above phrases.

For not publishing those abstracts, I offer no apologies. A journal's prime obligation is to inform and educate its readers. This in no way detracts from its other major obligation, to publish relevant submitted articles or abstracts from authors. In fact, these two obligations are mutually complementary.

Help is always available from the Editor. Indeed, I will be actively involved in the planning of next year's ASM in Wellington. I will look at submitted abstracts and, if appropriate, suggest ways for you to improve and make it more informative. In no way will your abstract be rejected for presentation at the ASM. It will, however, not be published in the following Journal issue if it does not meet the Editor's simple and basic requirements. In next year's March issue there will also be a sample abstract published that will show, what a good abstract should look like.

I look forward to receiving, and publishing, good quality abstracts of presentations at next year's ASM in Wellington. Hopefully, this figure will be 100%.

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Determination of *Chlamydia trachomatis* prevalence in asymptomatic male military personnel in New Zealand: comparing performances of COBAS Amplicor and Abbott LCx Chlamydia detection systems for urine specimens

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Abstract

Aims To establish the prevalence of *Chlamydia trachomatis* in 200 asymptomatic male army personnel and to evaluate nucleic acid amplification tests commonly used for the detection of *C. trachomatis* in the community.

Methods Two commercially available nucleic acid amplification (NAA) tests (Roche Amplicor™ and Abbott LCx™) were used to test 200 early morning urines collected from male New Zealand Army personnel for the presence of *C. trachomatis*. Discrepancies were resolved by use of polymerase chain reaction to test for presence of *C. trachomatis* Major Outer Membrane Protein (MOMP).

Results Both kits performed satisfactorily, giving an overall prevalence for C. trachomatis carriage of 4.0% in the study population. In all, there were three discrepant results. These discrepancies were resolved by PCR to the MOMP.

Conclusions NAA methods are a reliable method of screening urine samples from at-risk populations. Strategies aimed at controlling *C. trachomatis* in the general population should also include screening of at-risk males.

Key words: *Chlamydia trachomatis*, polymerase chain reaction, prevalence, screening, urine

Introduction

C. trachomatis is the most common cause of laboratory-diagnosed sexually transmitted disease in New Zealand and other industrialised countries (1,2). Some studies estimate up to 50% of infections in males are asymptomatic (3,4) and if left untreated may lead to epididymitis (4). *C. trachomatis* causes 40% to 50% of non-gonococcal urethritis in heterosexual males (5). Up to 70% of women with endocervical infection are asymptomatic (6). Common sequelae of untreated infection in women include pelvic inflammatory disease, ectopic pregnancy, tubal scarring, and infertility (3). When diagnosed, *C. trachomatis* infections can be easily treated and cured.

Available evidence indicates that chlamydia screening and treatment not only reduces the prevalence of lower genital tract infection, but also decreases the incidence of costly complications, such as pelvic inflammatory disease (4).

Sensitivity and specificity of molecular techniques in the detection of chlamydial infections are significantly higher than conventional techniques (7-9). Semi-automated molecular amplification techniques such as the Abbott LCx™ ligase chain reaction (LCR) system (10) and the Roche COBAS Amplicor™ polymerase chain reaction (PCR) system (11) have been successfully introduced for the diagnosis of *Chlamydia trachomatis* infections.

Present practice within the New Zealand Defence Forces is to test males presenting with a history of exposure to STDs, or suggestive symptoms. Females are generally screened when they have a cervical cancer smear. Asymptomatic males are not routinely screened, allowing for the possibility of an undetected pool of carriers within the community.

The aim of our study was to determine the prevalence of *C. trachomatis* in an asymptomatic male military population in New Zealand, and to compare the performance of the Roche Cobas Amplicor™ and Abbot LCx™ test methods for the detection of *C.trachomatis* infections using urine specimens.

Materials and methods

Specimen collection

A total of 200 early morning urine specimens were collected from male personnel from Burnham Camp (n=65) and army recruits from Waiuoru Military Camp (n=135). Ethical Committee approval was obtained from the Canterbury Ethical Committee, and all subjects provided written informed consent. Subjects ranged in age from 17 to 35 years, and were recruited to the study on a voluntary basis. Samples were frozen on site, and transported to the laboratory where they were stored at -20°C for a maximum of one month prior to analysis. Subjects who reported a history of recent symptoms were excluded from the study. Confidentiality was maintained at all times.

Chlamydia trachomatis testing

Roche Diagnostic Systems kindly donated Cobas Amplicor™ kits and Abbott Diagnostics kindly donated LCx™ kits for the purposes of the study. Barrier tips were used at all times during pipetting when performing assays.

Cobas Amplicor™ PCR method

The Cobas Amplicor™ PCR was carried out according to the manufacturer's instructions. Aliquots of wash buffer (0.5ml) were added to 1.5ml microfuge tubes. The urine specimens were thawed and mixed thoroughly and a 0.5ml aliquot was added to the respective microfuge tube. The mixture was vortexed and incubated at 37°C for 15 minutes followed by centrifugation at 12,000 g for five minutes at room temperature. The supernatant was discarded immediately by gently pouring off into a discard container and then blotting each tube on a separate section of absorbent paper to remove residual traces. After the addition 250µl of lysis buffer the specimens were vortexed and then incubated at room temperature for 15 minutes.

Two hundred and fifty (250) µl of specimen diluent was added to each sample, each sample was vortexed, centrifuged for 10 minutes at 12,000 g and stored frozen at -20°C prior to amplification. The prepared specimens were thawed, vortexed and re-centrifuged prior to addition to the PCR reaction tubes containing master mix. Controls were prepared concurrently on the day of testing.

Specimens and controls were then amplified and detected on the Cobas Amplicor™ instrument. An internal control was used for each specimen in order to detect any inhibition present. The COBAS Amplicor PCR detects *C. trachomatis* plasmid DNA.

Abbott LCx™ LCR method

The LCx™ test was performed according to the manufacturer's instructions. Urine specimens were thawed and mixed thoroughly and a 1.0ml aliquot was centrifuged at >9,000g for 15 minutes in a 1.5ml microfuge tube. The supernatant was removed with a disposable pipette within 15 minutes of centrifugation. One ml of resuspension buffer was added to the pellet. The specimen was vortexed and then heated in the dry bath at 97°C for 15 minutes. The specimens were then either stored frozen at -20°C prior to amplification and detection or added immediately to the amplification vials containing the LCR mixture (four oligonucleotides specific for the *C. trachomatis* plasmid, DNA ligase, polymerase' NAD, Mg^{2+}, dCTP, and dTTP in a pH 7.8 buffer). Two negative controls and two calibrators were included in each run. After amplification, the specimens were pulse-centrifuged before detection in the LCx™ analyser using an MEIA assay. The LCx methos also detects *C. trachomatis* plasmid DNA. Specimens showing a rate above that of the negative control average but less than the cutoff value were repeated using a double-wash technique during sample preparation to remove possible inhibitors.

MOMP analysis

Samples identified as *C. trachomatis* positive or negative by both PCR and LCR were defined as true positive or true negative. Discrepant samples, (ie PCR positive and LCR negative or alternately PCR negative and LCR positive) were subjected to rapid, real-time PCR to the Major Outer Membrane Protein (MOMP) of *C. trachomatis*. Reactions were performed on the LightCycler™ (Roche Molecular Biochemicals) using the SYBR Green I (SGI) format. SGI is a fluorescent dye that binds preferentially to double-stranded DNA. The accumulation of DNA (and SGI fluorescence) during the PCR is followed on-line as the PCR progresses.

DNA was extracted from 1ml and 2ml aliquots of each urine sample using the High Pure PCR Template Preparation Kit (Roche Molecular Biochemicals). The following pre-treatment was used: the cell pellets were resuspended in PBS, centrifuged and the supernatant discarded. Two hundred (200) µl fresh PBS was added and the pellets resuspended again. One hundred and fifty (150) mg of lysosyme was then added and the manufacturer's instructions for bacteria followed from this step. DNA was eluted in 100µl of the supplied elution buffer (10mM Tris).

PCR reactions were performed in a 20µl reaction and consisted of 5µl template eluate, 2mM $MgCl_2$ (final concentration), 0.35µM each primer and 2µl LightCycler DNA Master SYBR Green I which contained the Taq, dNTPs, 1 mM $MgCl_2$, buffer and SYBR Green I dye (Roche Molecular Biochemicals). Primers (obtained from MWG Biotech, Germany) were as described by Dutilh and colleagues (12) and have been used previously for PCR confirmation of *C. trachomatis* (13). Reactions were 'hot-started' by addition of 100nM of the TQ30 aptamer during the reaction set-up (14). The PCR program consisted of a 2 minute initial denaturation followed by 45 cycles of heating to 95°C for 0 (zero) seconds (denaturation), cooling to 62°C for five seconds (primer annealing) and heating to 72°C for 10 seconds (extension). Such short cycle times are possible due to the capillary format and high ramp speeds of the LightCycler. This allowed the PCR and detection to be performed in only 25 minutes. The presence of specific product (SGI will also bind to non-specific products such as primer-dimers) was determined by slowly heating the products and determining the temperature at which the DNA was denatured (shown by a decrease in SGI fluorescence). This is most conveniently displayed as a melting peak. Previous work (unpublished observations) indicated a melting peak of approximately 86°C corresponded with the presence of the desired 129bp PCR product.

Figure 1 Real-time amplification curves for 129bp MOMP product from *Chlamydia trachomatis*

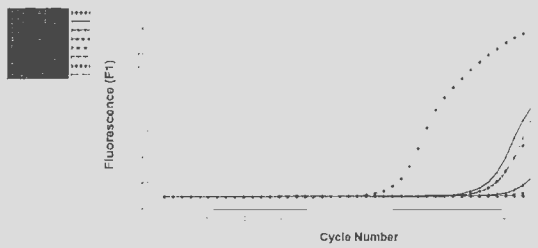
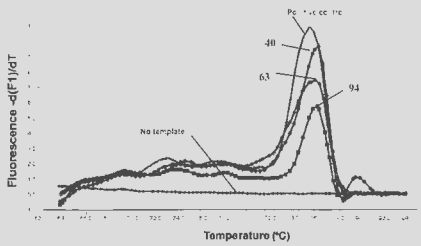


Figure 2. Melting peaks of MOMP products



Amplification curves (Figure 1) with specific melting peaks (Figure 2) were obtained for all 1ml and 2ml samples. However, amplification was not always obtained in successive runs for two of the three 1ml samples (data not shown), while products were always generated for the 2ml aliquots.

Results

Prevalence and patient demographics

The overall prevalence was 4.0% in this group of asymptomatic men. The age profile of the two groups is presented in Table 1.

Table 1. LCx™ and COBAS Amplicor™ test results, age profiles

	Total	Average Age	<25y	<20y	Lowest Age	Highest Age
Confirmed Positive	8	22	75%	12.5%	18	27
Confirmed Negative	192	21	83%	51%	17	38
All Participants	200	22	83%	49%	17	38

COBAS Amplicor™ PCR detected no inhibition in all samples. The Abbott LCx™ method did not contain an internal control and therefore no overall assessment can be made on the presence or absence of inhibition in the test samples with this method. Five urine specimens showed rates significantly above the LCx™ negative control average. In this event it is our laboratory policy to repeat the test using a double-wash technique. Four of the urines were negative on repeat LCx™ test, and one was positive. The positive sample was also positive for Amplicor™ PCR. The original negative LCx™ result may have been due to inhibitory substances present in the urine sample.

Six out of 200 urine samples were positive for Amplicor™ PCR (3.0%) and 7 out of 200 samples were positive for LCx™ (3.5%). There were 3 discrepant results..Two of the 8 positive results were positive for LCx™ and negative for Amplicor™ PCR. One of the 8 was positive for Amplicor™ PCR and negative for LCx™. Eight out of 200 urine samples were positive either by Amplicor™ PCR or Abbott LCx.™

Discrepancy analysis

In each of the three discrepant samples, LCx™ and COBAS Amplicor™ were repeated using a fresh aliquot of urine. In the case of LCx™, each sample was given a double wash during sample preparation to remove possible inhibitors. In one case the repeat LCx™ was negative. MOMP PCR analysis was performed on all three discrepant results with a 1ml and 2ml aliquot of urine. One out of three was positive with the 1ml aliquot of urine. All three were positive with the 2ml aliquot. A true positive was therefore defined as any sample showing a positive LCx™ or Amplicor™ test which was confirmed by either the 1ml or 2ml MOMP PCR test. Resolution of discrepant samples showed 8 samples out of 200 to true positives giving an overall prevalence of 4.0% (Table 2).

Table 2. Discrepancy analysis results

Discrepant Analysis Number	LCx™	LCx™ Repeat	COBAS Amplicor	COBAS Amplicor Repeat	MOMP PCR 1 ml	MOMP PCR 2 ml
1	-	-	+	+	+	+
2	+	+	-	-	-	+
3	+	-	-	-	-	+

Our results indicated that the overall prevalence of *C.trachomatis* in this study after resolution by discrepant analysis is 4.0%. Discrepant analysis sample number 1 contained substances that were inhibitory to the LCx™ assay. Discrepant analysis sample numbers 2 and 3 probably contained low numbers of the *C.trachomatis* elementary bodies within the samples and hence the spurious detection rates. When using the Abbott LCx™ test on urine samples, it is important to retest samples showing significant activity above the negative cutoff point and below the positive cutoff point after a double washing step.

Discussion

Chlamydia infection is a major public health problem. There is little information available on prevalence of chlamydia infection in the New Zealand population. Previously available tests have not been sufficiently sensitive and specific to apply to populations with low prevalence rates. Trauma and discomfort associated with specimen collection in males and females (especially when requiring cervical examination in girls less than 16 years of age) limits research initiatives, however newer NAA based tests are not invasive and are more acceptable to patients.

Samples sent to community laboratory test providers represent a highly selected population. In addition to symptomatic patients, samples may be collected for contact tracing, "test of cure", opportunistic screening, sexual health programmes, and the "worried well". A recent ESR report analysing laboratory data from the Waikato and Bay of Plenty) indicated an alarming increase in sexually transmitted disease rates. The report also noted that the majority of detected cases occur in patients between the ages of 15 and 29 years (15).

To our knowledge, this is the first study to look at the prevalence of *C. trachomatis* in an asymptomatic male population in New Zealand. Our study reports a prevalence of 4.0% in 200 males. This rate is similar to that found in recently reported study in a Dutch (asymptomatic) military population, where a prevalence of 4.1 % was recorded (16), and more than that reported (2.8%) in a similar recent study on asymptomatic Dutch males (17).

Our study has made no attempt to screen for independent risk factors. Other studies have identified age (>25 years), lower socio-economic status, and number of partners as risk factors. A recent article analyzing trends in gonorrhoea infections in the USA closely linked State alcohol policy with gonorrhoea infection. Legislated increases in the minimum legal drinking age increases were followed by a relative proportionate decrease in the gonorrhea rate in 15 -19 year olds (18). Alcohol intake may thus also represent a significant risk factor in *Chlamydia trachomatis* infection.

The chronic complications of chlamydia infection impacts disproportionally on the female population. The consequences of the untreated disease include PID, infertility, and ectopic pregnancy. Advances in molecular diagnosis of chlamydia infection present health workers with a unique window of opportunity to attempt to control and reduce the rate of new infections. The ease of specimen collection ensures patient acceptance, therefore facilitating early diagnosis as well as exhibiting high specificity and sensitivity.

In New Zealand, public awareness of chlamydia as a sexually transmitted disease remains low. In our opinion there is an educational component in chlamydia control that is not being fully exploited, particularly amongst younger age groups. An overall strategy for chlamydia control in the at-risk population may result in saving healthcare dollars further downstream. One randomized trial indicated that it was possible to reduce PID in high-risk females by 50% in 12 months, demonstrating positive health outcomes that considerably outweighed associated test costs (19).

Although there are direct and obvious benefits in screening female patients, the existence of an asymptomatic male reservoir, as demonstrated in our study, represents an otherwise undetected source of infection and brings up the question of male screening programs. Previous studies of the economic impact of mass screening have indicated that molecular testing is cost effective in populations with prevalence rates above 3% (20).

Strategies developed to control chlamydia infection in the general population should include a component aimed at addressing asymptomatic carriage in males. Our study demonstrates that testing first void urine from an at-risk population simplifies screening, and in our opinion meets Jungner and Wilson's criteria Table 3) for a workable screening programme (21,22).

Table 3. Jungner Wilson criteria for disease screening in a population

1	Condition sought an important public health problem.
2	Accepted treatment for patients with disease.
3	Facilities for diagnosis and treatment available.
4	Recognisable latent or early symptomatic stage.
5	Suitable test or examination.
6	Test acceptable to population.
7	Natural history of condition adequately understood.
8	Agreed policy on whom to treat.
9	Costs (including those of diagnosis and treatment) economically balanced in relation to expenditure on medical care as a whole.
10	Continuing process not "once and for all" project.

In conclusion, this study has found an overall prevalence rate of 4% for *C.trachomatis* in asymptomatic male New Zealand Army personal by the use of nucleic acid amplification tests for the detection of *C.trachomatis* in first-catch urine samples. Both Abbott LCx™ and Roche Amplicor™ testkits were found to be suitable techniques for the detection of *Chlamydia trachomatis* carriage in the study population.

Acknowledgments

We wish to thank all involved who helped with the organization and collection of samples particularly the staff from both Burnham and

Waiouru medical treatment centres. We are very grateful for the technical and scientific support provided by Southern Community Laboratories and the Canterbury Geriatric Medical Research Trust, and we thank Roche Diagnostics NZ and Abbott Diagnostics NZ for supplying reagents and equipment. Lastly we wish to thank the participants for volunteering to be a part of this study.

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NZIMLS President's Report 2001

The past year has been very successful for the NZIMLS. From the last scientifically and fiscally successful conference to this conference, which promises to deliver a great scientific and social program, the year has been one of meeting, particularly, our goals of continuing education needs.

It does require extra effort and commitment to become involved in extra tasks such as a conference committee, contributions within a Specialist Interest Group or other facets of voluntary professional work.

Year 2001 is International Year of the Volunteer, so it is very timely to remember, acknowledge and thank all the volunteers within our professional body that contribute to make our profession successful. Thank you.

The Annual Report has been circulated and contains the Annual Report, Balance Sheet and Annual Accounts. However I take the opportunity to highlight just a few of the past years progresses to you. Operationally Council has reviewed and where necessary, further refined mechanisms that ensure *"best value for membership and sponsorship dollar"* and *"most effective services and opportunities to members"*. We are all busy people and the guidelines, job descriptions and various contracts streamline efforts into coordinated results.

Communication is and always will be a key ongoing focus, with multiple forms. For example:

Scientific Journal. Rob Siebers, our current Editor, in his annual report makes his traditional and sincere offer of assistance, to would-be authors on behalf of the whole Editorial Team. This voluntary resource is as yet, still under-utilized.

Special Interest Group meetings and contributions to the Scientific Journal are again a voluntary activity providing communication vehicles and opportunities for continuing education and Continuing Professional Development (CPD) points. It is tremendous to see the growth in this area and as presenters mature, we should increasingly see progression to presentation at the Annual Scientific Meeting and/or publication in our Scientific Journal.

www.nzimls.org.nz: This site was designed and developed using membership funds, coordinated by Council volunteers, specifically Chris Kendrick, to present our professional body on the Internet. The return on this investment is real:

- with members and potential members utilising services
- reprints of journal articles requested from overseas
- links to the conference website

All being valued.

The most challenging area of communication remains those who have yet to grasp the differences between the roles and responsibilities of the NZ Laboratory Workers Union - wage negotiation. Medical Laboratory Technologists Board - registration, public watchdog, maintenance of standards etc. New Zealand Institute of Medical Laboratory Science whose objectives include :

- To establish and promote standards appropriate to the profession of Medical Laboratory Science
- To improve the standards, status, education and training of members of the profession
- To encourage the publication of material relating to, or associated with Medical Laboratory Science
- To provide counsel, advice and representation on matters pertaining to Medical Laboratory Science
- To do all things considered necessary for, or beneficial to the Institute or its members

In conclusion, the NZIMLS is the professional body of Medical Laboratory Scientists, run by volunteer Medical Laboratory Scientists and administered by one paid employee. It provides opportunities through voluntary effort for members to uplift and benefit from.

Anne Paterson
President

Minutes of the 57th AGM of the NZIMLS. Auckland, Wednesday, September 2001

Chair

The President (Mrs A Paterson) presided over the attendance of approximately 32 members.

Apologies

R Siebers, J Deans, L Milligan, S Melvin

Proxies

A list of 3 proxies were read by the Secretary.

Minutes

Motion:
Moved S Benson, seconded T Mace
That the minutes of the 56th Annual General Meeting held on Thursday 17th August 2000 be taken as read and accepted as a true and correct record

Carried

Business arising

IAMLT

- Questioned from the floor as to how much it costs the NZIMLS to be a member of the IAMLT? THis is approximately NZ$1,400
- It is anticipated that there are 25-30 countries who are IAMLT members.

Motion:
Moved D Reilly, seconded W Wilson
That the NZIMLS retains its membership of the IAMLT.

Discussion:

- D Reilly spoke to the motion. The IAMLT is the only international operation working in at an international level. There is no other association for technologists around the world. There have been a number of people involved with the IAMLT over the years and there are a number of membership benefits. The IAMLT have taken on board the NZIMLS document on Near Patient Testing, the MLTB competency document and the NZ Universities courses are used as reference material for a lot of countries. D Reilly feels that technologists are doing a lot of good work in New Zealand which is of benefit internationally and that we have a lot to offer the world. If the NZIMLS remains a member, then we can still have a voice and presence. D Reilly acknowledged that the industry is shrinking and that the IAMLT have been slow to react to this. The IAMLT is now working to improve their financial situation.
- A Bunker questioned if the NZIMLS bank account is healthy and if it would support the membership subscription of the IAMLT. T Rollinson confirmed that the NZIMLS is in a healthy financial situation. W Wilson supported the motion to remain a member of the IAMLT. He felt that New Zealand as a small country gets recognition for being involved and that we have a lot to contribute. Feels that the IAMLT have to make changes and that we can only help make these changes from within.
- A Fitchett questioned if there was a financial risk to the NZIMLS remaining as members of the IAMLT. Legal advice was sought in 1999 and the general legal comment was that the NZIMLS is not at risk.

The motion was put to the membership and carried with two votes against.

Phlebotomy

- The President acknowledged the work and commitment that Ailsa Bunker had put into organising and participating in the Phlebotomy workshop and the huge undertaking to for further development. The workshop had opted to form a working party to work through the NZIMLS. Minutes of the workshop will be reported back to Council.

Remits

Motion:
Moved S Benson, seconded W Wilson
That Policy Decision Number 1 be reaffirmed

Policy Decision No 1 (1971): That all committees and meetings convened under the auspices of the New Zealand Institute of Medical Laboratory Science (Inc) be subject to a standard reference of parliamentary procedure and that this a 'A Guide for Meetings and Organisations' by Renton.

Carried

Motion:
Moved T Mace, seconded R Bluck
THAT Policy Decision Number 2 be reaffirmed

Policy Decision No 2 (1989): That all persons wishing to undertake any examinations offered by the New Zealand Institute of Medical Laboratory Science (Inc) shall at the time of application and the taking of the examination be financial members of the Institute.

Carried

President's report

Motion:
Moved A Paterson, seconded T Mace
That the President's Report be received.

Carried

Annual report

Motion:
Moved W Wilson, seconded J Clark
That the Annual Report be received and adopted.

Carried

Financial report

Moved T Rollinson, seconded L Brennan
That the Financial Report be received and adopted.

Carried

- W Wilson acknowledged the commitment of T Rollinson to get a positive balance.

Election of officers

The following members of Council were elected unopposed:

President		L Milligan
Vice President		C Kendrick
Secretary/Treasurer		T Rollinson
	Region 1 Representative	S Benson
	Region 2 Representative	T Mace
	Region 3 Representative	C Green
	Region 5 Representative	A Buchanan

An election was necessary for the position of Region 4 Representative. The election result was:

J Mills	2
J Sheard	13
J Deans	25

J Deans was declared the Region 4 Representative.

Awards

The award winners were announced and the awards where possible were presented by the President:

Qualified Technical Assistant awards

Clinical biochemistry	Lesley Graybill, Medlab Timaru
Medical cytology	Bettina Buch, Greenlane Hospital
Haematology	Helena Woods, Wellington Medical Laboratory
Histology	Jean Fannin, Medlab Bay of Plenty
Immunology	Deborah Armstrong, Canterbury Health Labs
Microbiology	Kristine Isakow, Diagnostic Medlab
Mortuary hygiene & technique	Grant Milne, Wellington Hospital
Transfusion science - blood products	Carina Bell, New Zealand Blood Service
Virology	Natalie Currie, Waikato Hospital

Honoraria

Motion:

Moved T Mace, seconded L Manuel

That no honoraria be paid.

Carried

Auditor

Motion:

Moved T Rollinson, seconded A Calvert

That Hillson, Fagerlund and Keyse be appointed as the Institute's auditors.

Carried

General business

Phlebotomy group

- A Bunker suggested that Council consider a Phlebotomy Assistants membership category as these people are keen to be involved. Noted that the facility for these people to be involved with the NZIMLS is already there in that they can become a non practising member.
- Suggested a Phlebotomy SIG. The NZIMLS will support a Phlebotomy SIG.

Creditionaling

- Questioned if anything had happened in light of the Gisborne affair? A Paterson advised that she had attended a meeting with the Ministry of Health regarding creditionaling. This must be a partnership between the employer and the profession. Noted also that the Ministry is very keen to see the HPCA bill go to Parliament before the end of the year.

- P Saxby suggested visiting the Ministry of Health website at www.excu.govt.nz for updates on creditionalling and the HPCA bill.

Venue for the year 2002 Annual Scientific Meeting

- To be held in Wellington. R Siebers is the conference convenor and R Yee the scientific convenor.
- A venue for the 2003 AGM and the 2004 ASM was not found. Council will follow-up.

Meeting closed at 6.30pm

Abstracts of presentations at the NZIMLS ASM, Auckland, September 2001

The biosynthetic pathway for blood group related glycoconjugates in the human gastrointestinal tract. A map of pathogen receptors and insights into ABO.

Professor Stephen Henry, Director Glycoscience Research Centre, Auckland University of Technology

In order to further understand the complexities of biosynthesis and to define the blood group structures available as receptors for microorganisms (both commensals and pathogens), we isolated blood group related structures from the small intestine of individuals with different blood types. Using immunochemistry, proton NMR and mass spectrometry (MALDI and Q-Tof MS/MS) we have resolved the structures present. With this structural information we have been able to construct a biosynthetic pathway for the blood group related glycoconjugates in the human GI tract. It was found, as previously postulated, that elongation and branching of glycoconjugates appear to be regulated by blood group glycosyltransferases. In individuals deficient in, or having inefficient blood-group related fucosyltransferases, generally more extensive elongation and/or branching occurs than is found in those with normal fucosyltransferase activity.

Furthermore, it appears that the type of precursor chain may also determine whether the precursor chain will be elongated or branched. The biosynthetic control/modification of the precursors is to some extent under the influence of the Lewis and Secretor blood group fucosyltransferases although other non-blood-group related glycosyltransferases can also utilise these precursors. There is different biosynthesis utilisation of the precursors which has significant biological implications as individuals with different blood types make differing amounts (ranging from almost nothing to very large amounts) of type 1 and type 2 based structures. The consequences are as yet unresolved, although of the surprisingly limited range of structures made, many of them resemble tumour antigens and some are identical to bacterial LPS's.

Determining the structures of the blood group related glycoconjugates in the GI tract has enabled the development of a chart of potential receptors to which microorganisms may bind. With this chart plus methodical and accurate disease association surveys we may now be able to fathom the complexities of how microbes interact with the glycoconjugate interface and how this contributes to the next stages of virulence and pathogenesis. This may lead to the design of effective ways to prevent and/or disrupt the interaction, concluding as a therapy to the disease.

Non-myeloablative stem cell transplantation and the graft-versus-leukaemia phenomenon - a translational research approach.

Dr AP Schwarer, Bone Marrow Transplant Programme, Alfred Hospital, Melbourne, Australia

Allogeneic stem cell transplantation (alloSCT) offers the chance of cure for some patients with malignant and non-malignant haematological diseases. AlloSCT is restricted to younger and fitter patients because the risk of significant transplant-related morbidity and mortality. A prime goal of many researchers in this field is to decrease the risks of alloSCT while maintaining or enhancing the curability of the underlying malignancy.

Considerable in vitro evidence in humans and in vivo evidence in animal models suggests that a graft-versus-tumour (GVT) phenomenon is, at least in some instances, important to the success of alloSCT. Recent in vivo evidence in humans strongly supports this concept.

The evidence alluded to above has led to the concept of non-myeloablative (mini-) alloSCT: lower doses of chemo/radiotherapy are used to decrease toxicity but ensure engraftment, and hope that the GVT phenomenon will be adequate to prevent relapse. Many studies in older, less fit patients now indicate that the mini-alloSCT is safer than a standard alloSCT and that some patients can achieve long-term disease-free survival, but that graft-versus-host disease (GVHD), infection and relapse remain significant problems. Some workers, including ourselves, administer routine donor lymphocyte infusions to these patients to promote a GVT phenomenon although the risk of such an approach is an increase in the incidence and severity of GVHD.

To improve the safety and efficacy of mini-alloSCT, our group is currently developing techniques using donor-derived dendritic cells and haematopoietic-specific polymorphic minor histocompatibility antigens produced in insect cell lines to "educate" donor T cells to recognise patient haematopoietic (and hence, haematopoietic tumour cells) cells. These "educated" T cells will be infused into patients at intervals after mini-alloSCT to generate a potent GVT response without significant GVHD.

Inborn errors of metabolism - why Biochem 101 was important

Dr Callum Wilson, Metabolic Paediatrician, Starship Hospital, Auckland Healthcare

Inborn errors of metabolism are a rapidly growing group of disorders caused, in general, by a deficiency of an enzyme. The conditions are genetic and mostly of autosomal recessive inheritance. The severe forms tend to present in early childhood. To simplify matters they can be divided into disorders of small molecule intoxication, small molecule energy production, large complex molecules and mitochondrial disease.

Small molecule intoxication disorders tend to present early in life. Due to a genetic defect in an enzyme, small molecules such as ammonia, amino acids or organic acids accumulate to massively elevated levels. These molecules are toxic to the brain and liver. The children often present therefore with lethargy, vomiting and hepatitis. This clinical picture is often confused with sepsis. A correct diagnosis, and therefore treatment, relies on the measurement of these compounds in urine and plasma.

Defects in small molecule energy production often present during an inter-current 'viral' illness when the child is exposed to 'catabolic stress'. The two main groups are disorders of fatty acid oxidation and defects of glycogen breakdown (glycogen storage diseases). Recent advances have made the diagnosis of these conditions relatively easy. Treatment is based on ensuring the child does not undergo prolonged periods of starvation.

Children with large complex molecule disorders (lysosomal storage diseases, peroxisomal diseases) classically present with slowly progressive

symptoms of developmental regression, hepatosplenomegaly and other organ involvement. Diagnosis is usually made by direct enzymology. Treatment, in general, is disappointing.

Mitochondrial disease can present at any age, in any organ and with any symptom. Usually but not invariably the patient will have some form of CNS involvement. The diagnosis often requires a muscle biopsy for histochemistry and enzymolgy. The latter performed only in specialised laboratories. Treatment is disappointing.

The relevance of the blood film in the fully automated haematology laboratory

Gillian Rozenberg, Haematology Department, Prince of Wales Hospital, Randwick, NSW, Australia

Fully automated blood analysers provide accurate estimation of cell numbers as well as reliable observations on cell patterns. This information is then fed into an attached computer with an appropriate software program designed to analyse the findings and interpret them. This works well when the count is within the normal reference range and a result can be printed and sent out. In instances where the count or cell pattern is outside the normal reference range, a blood film is needed and an experienced morphologist to interpret the findings is essential. Automated counters can be programmed to make and stain a blood film in all instances whether the findings on the count or pattern are normal or abnormal. Looking at the blood film in normal results may be omitted. In the abnormal situation, the blood film is used to complement the findings obtained by the automated counter.

Blood film examination is an indispensable part of the investigation in the following:

(1) When the analyser displays an atypical lymphocyte pattern

(2) When looking for cytoplasmic inclusions in granulocytes

(3) When the platelet count is falsely elevated due to the presence of small red cell fragments or when it is falsely reduced during the recovery phase of thrombocytopenia when young platelets may be large and not counted by the analyser

(4) When an interference red cell flag or white cell flag is registered.

(5) A blood film is also essential to diagnose malaria.

Prions and blood products

Dr Eric Uren, CSL Bioplasma, Melbourne, Australia

Although there is no evidence of blood borne transmission in humans of the variant form of Creutzfeldt Jacob Disease (vCJD), the precautionary step of UK donor deferral has been implemented in Australia and New Zealand to reduce the theoretical risk of infection from blood or blood products.. To demonstrate the safety of plasma derived products, the plasma fractionation industry has ongoing evaluation programs to determine the capacity of the purification processes to eliminate infectious TSE agents or the proteinase resistant PrP^{res} marker.

Studies to evaluate the capacity of a number of partitioning steps to remove the abnormal isoform of scrapie prion (PrP^{sc}) have been undertaken by CSL Bioplasma. PrP^{sc} was used as a model for the vCJD prion and scale down studies were performed at Q-One Biotech Ltd in Glasgow utilising the Western blot methodology (WB). The experimental studies employed the microsomal fraction of hamster brain from animals infected with hamster-adapted scrapie agent strain 263K. The PrP^{sc} form in the microsomal fraction is thought to be the most suitable to model that which may be present in plasma, representing broken membrane fragments which are the potential contaminants of human blood. The detection of PrP^{sc} utilising WB analysis of the Proteinase K-resistant form of PrP is considered to be a suitable screening technique in that it parallels the infectivity of the scrapie agent. Substantial reduction of the PrP^{sc} has been demonstrated in the ion exchange and precipitation steps examined to date. These results are similar to published reports which show that precipitation, chromatographic and filtration steps can contribute to significant removal of PrP^{sc}. Studies are ongoing to understand further the capacity of the purification processes to partition the prions away from the target proteins.

"An interesting Corynebacterium sp"

Sue Paviour, Microbiology Laboratory, LabPlus, Auckland Healthcare

Corynebacterium is a large genus of bacteria. They are Gram positive irregularly shaped bacilli with a high G+C content in their genome. There are 46 identified species, 31 of which are considered clinically relevant. Eighteen of these have been identified since 1995. (Funke and Bernard 1997 ASM manual.)

In recent years our Laboratories have isolated Coynebacterium species from breast tissues, abscesses and granulomatous mastitis. The significance of these isolates has been a recurring problem for us as Corynebacterium sp. are often normally resident on the skin. The literature was reviewed and a few case reports of different Corynebacterium species being isolated from patients with breast disease were found.

To answer the question of significance, patients in whom isolates of Corynebacterium species have been found from breast specimens between 1994 and 2001, were reviewed. A retrospective review of available organisms, including phenotypic identification by API CORYNE kit(bioMerieux), lipohilicity, catalase, and other biochemical tests, and 16s rRNA gene sequencing was undertaken. Case notes were reviewed for clinical details, risk factors, ethnicity, age and treatment. Histology reports of breast tissue if available was also reviewed. Gram stain, repeated isolation and other pathogens present were also recorded. Antimicrobial testing by breakpoint MIC was undertaken. Thirty-eight separate isolates of Corynebacterium species were isolated from 24 women. Sixteen of these isolates were available for full identification and gene sequencing. Twelve of these were identified as *C. kroppenstedtii*. This organism was described in 1998 on the basis of a single unique isolate from sputum.

Lipophilicity and a positive aesculin reaction distinguish it from other Coynebacterium sp. 11 women had either an excision specimen or multiple specimens sent for histology, all of these patients had granulomas in at least 1 specimen. In 7 of the 11 patients Gram positive bacilli were detected in one of their histological specimens.

Prospective studies are needed before an aetiological role for this organism in breast pathology can confidently be postulated. We have so far raised more questions than answers.

Haemovigilance at Middlemore

M.J. Dewse, Transfusion Review Nurse, Middlemore Hospital, South Auckland Health

Current management practices acknowledge the value of assessment and evaluation in the delivery of quality service. The American Association of Blood Banks recommends the monitoring of transfusion practice and peer review to promote efficiency and excellence in the practice of transfusion medicine. Hospitals in other countries have found it advantageous to employ a registered nurse with specific responsibility in this area.

With the support of the hospital transfusion committee, the Blood Bank at Middlemore Hospital appointed a Transfusion Review Nurse in June 1997. The job description for this position included responsibility for monitoring of transfusion practices, promoting staff awareness of policies and protocols related to the use of blood and blood products and response to queries from patients and their families related to this area of treatment.

This role has provided an interface between the laboratory and clinical staff and become a means to identify and eliminate problems, which undermine good clinical practice. It has become a tool for haemovigilence, improved standards of documentation, provided staff education about transfusion medicine and been useful in providing information and support for patients receiving blood products.

The Otago experience: consolidating and confirming the program

Dr Chris Lovell-Smith, Course Director BMLS, University of Otago, Dunedin

Since the Otago program began, we have graduated 175 students - an average of 25 per year. We are now an established part of the well-known health sciences learning environment at the University of Otago.

Our program was extensively reviewed earlier this year, and significant changes are underway as a result. Look out for these possibilities:

- Easier entry to the degree program for working scientists
- New papers and more options at undergraduate and postgraduate level
- Increasing emphasis on research as a part of a worthwhile career
- Revised first-year and admission options
- Improved facilities and staffing
- Revised assessment procedures.

Don't expect changes to:

- Otago's established reputation for academic excellence
- Our fully accredited status
- The 'scarfie experience'.

Testing for recombinant EPO at the Sydney Olympics

Graham J Trout[1], Kerry R Emslie[1], Christopher J Gore[2], Allan G Hahn[2] , Chris Howe[1], Francoise Lasne[3], Robin Parisotto[2] and Rymantas Kazlauskas[1]. [1]Australian Sports Drug Testing Laboratory, Australian Government Analytical Laboratories (ASDTL), Sydney, Australia, [2]Department of Physiology, Australian Institute of Sport (AIS), Canberra. Australia, [3]Laboratoire de Depistage du Dopage, Creps, Paris, France.

Evaluation of the results of the 1999 EPO administration trial carried out in collaboration with the Australian Institute of Sport (AIS) indicated that there was a good possibility of detecting EPO abuse by using a combination of blood and serum parameters. In order to confirm the validity of these results a large scale international validation study (EPO2000) was carried out jointly funded by the International Olympic Committee (IOC) and the Australian Government. The results of these studies were presented to a panel of scientific experts convened by the IOC in late July 2000. As a result of this meeting a decision was made on the 1st August to implement testing for EPO at the Sydney Games in September.

The approved test was a combination of the indirect blood test developed by the ASDTL and the AIS and a direct urine test developed by our colleagues at the Paris IOC laboratory (LNDD). Blood and urine samples were taken from over 300 targeted endurance athletes prior to competition, with the aim of detecting and deterring EPO abuse.

Brave new disease management world? The Northern region hepatitis screening programme

Dr Chris Bullen, Project Director, Public Health Medicine Specialist, Auckland District Health Board

Aiming to test around 175 000 people from high-risk populations, this two-year programme has broken new ground in a number of ways: these include a governance body based on a treaty relationship between a crown health agency and an iwi partner; recruitment of participants by means of opportunistic invitation at GP surgeries, combined with community networking and targeted media campaigns; and the use of sophisticated electronic links to capture data for management and monitoring. One year into the program much has been achieved: as at July 1 2001 more than 100 primary care sites were actively screening participants in Auckland and Northland and 30 000 people had been tested, 8% of whom were found to be carriers.

Early prediction of outcome by Troponin I, Troponin T and CKMB in acute chest pain without electrocardiographic evidence of ischaemia or infarcton

Lyndsae Wheen[1], Nigel Moody[2], Anthony McClelland[1] and D Ross Boswell[1]. Departments of Chemical Pathology[1] and Medicine[2], Middlemore Hospital, Otahuhu, New Zealand

Background The cardiac troponins are useful in identifying patients with acute myocardial syndromes and delineating a poor prognostic group amongst these. We set out to determine whether the troponins would be useful in identifying patients at low risk of a myocardial event, and to see if they might also give prognostic information in this low risk group.

Methods Four hundred and thirty patients who presented with chest pain unexplained by trauma or abnormalities on chest x-ray or electrocardiogram (ECG) were followed for 90 days. We compared the usefulness of troponins T and I (TnT and TnI) and the mass of MB isoenzyme of creatine kinase (CKMB) measured at least four hours after the onset of chest pain to predict 90-day outcome in these patients.

Finding Within 90 days of presentation, 41 patients had a myocardial event and 387 did not. The specificities of TnT and TnI (0.98 and 0.97) were significantly ($p < 0.05$) greater than that of CKMB (0.87). Likewise, the positive predictive values of the troponins (0.80 for TnI

and 0.67 for TnT) were significantly higher than that of CKMB (0.33). The three markers did not differ significantly in terms of sensitivity or negative predictive value (NPV). Overall, NPV was 95% or greater for all three markers.

Interpretation As a proportion of people who suffered an adverse event had a below threshold marker, clinical history and examination must be carefully assessed and given due weight despite normal ECG and cardiac markers. Nonetheless, the high NPV of all three markers means that a patient with a nondiagnostic ECG and below threshold cardiac marker taken at least four hours after the onset of chest pain is highly unlikely to have an adverse event within 90 days.

Thalassaemia and haemoglobinopathies

Dr Stephen Brennan, Molecular Pathology Laboratory, Canterbury Health Laboratories, Christchurch

The changing pattern of emigration to New Zealand combined with the development of new technologies for the investigation of molecular disease has presented us with both an increased demand for the diagnosis of thalasaemias, and novel means for the detection of haemoglobinopathies.

Some 336 different mutations have now been identified as causing either (or (thal and we routinely screen for six of the more common α thal deletions ($\alpha^{3.7}$, α^{SEA}, α^{Med}, $\alpha^{20.5}$, $\alpha^{4.2}$, and α^{Phl}) using automated DNA isolation and PCR set up.

While standard haemoglobin electrophoresis and stability tests can be used to identify many common haemoglobinopathies such as Hb S and Hb E, the clinically important mutations that give rise to haemolytic anaemia and polycythaemia have been more difficult to detect or exclude. By and large, these mutations occur in the hydrophobic interior of the molecule and do not involve a change in charge; consequently they can not be detected electrophoreticaly. Almost every possible mutation will however produce a small change in globin mass and this can now be detected by mass spectrometry of whole haemolysate.

Electrospray ionisation mass spectrometry can be performed in about five minutes on less than 0.1µl of lysate and the masses of the haemoglobin chains determined to within one Da. This means mutations can't hide and that mass spectrometry should be employed at the initial investigation of a polycythaemia and not as a last resort after all the expensive cardio-respiratory, rubera vera and other assessment have been complete.

Involvement of Coagulation Gene Polymorphisms in Myocardial Infarction

Dr Neil Van de Water, Molecular Haematology, LabPlus, Auckland Hospital

The contribution of genetic and functional alterations in factors of the coagulation and fibrinolytic pathways to the development venous thrombosis is well established but their role in arterial thrombosis requires clarification. Most of the known and well documented risk factors for myocardial infarction are involved with the atherosclerotic process. The influence of major risk factors for myocardial infarction such as dyslipidaemia, smoking, hypertension and diabetes may be overwhelming making it difficult to assess the potential role of other risk factors such as coagulation pathway protein polymorphisms. Dividing patients after myocardial infarction into two groups, those with coronary stenoses (the majority) and those who have no coronary stenoses, may help determine whether any one, or a combination of thrombotic risk factors contribute and predisposes the development of acute myocardial infarction. In several studies elevated levels of haemostatic proteins such as prothrombin, factor V, factor VIII and factor VII have been identified as risk factors for venous thrombosis and are potential risk factors for the future development of stroke and myocardial infarction. Possible pathogenic roles for other thrombophilic risk factors, including inherited abnormalities/polymorphisms of plasminogen activator inhibitor-1, methylenetetrahydrofolate reductase, beta-fibrinogen, platelet glycoprotein IIb/IIIa, angiotensin II type 1 receptor, haemochromatosis gene, nitric oxide synthase, p22 phox of NADPH oxidase and angiotensin converting enzyme, have also been proposed.

We have found 3-4 fold increased frequencies of inherited haemostatic risk factors, Factor V Leiden and prothrombin variant G20210A, in young patients with angiographically normal arteries following myocardial infarction, compared to the frequencies of these factors in unselected survivors of myocardial infarction and the general population. Fibrinogen variants associated with increased levels of fibrinogen may also play a role in the development of myocardial infarction. Our data supports the potential importance to the pathogenesis of myocardial infarction of mutations / polymorphisms in genes encoding proteins involved in coagulation, and in particular the protein C pathway.

In search of the ideal H&E: myth or reality? H&E methods in use by histology laboratories in Australasia

AH Hopkins, HL Llewellyn, RCPA QAP Anatomical Pathology, Bentleight East, Australia*

Objective The aim was to survey 220 laboratories participating in the RCPA Anatomical Pathology Quality Assurance Program on the standard of quality of H&E staining over 4 years. To do this effectively involved setting up a standardised method for making a qualitative assessment of staining that enabled interlaboratory comparisons and intra-laboratory comparison. Trends in both improvement and deterioration were examined. Questions raised were: what type of haematoxylin and what type of eosin were used, whether progressive or regressive methods were used, and their relative effectiveness. Other questions raised were: how laboratories monitor the quality of H&E staining and if a H&E control is used in this process. Common problems and artifacts of H&E staining were also reviewed. Follow up material is offered to all laboratories.

Statement of findings Fifty multi-tissue blocks with a variety of tissue components was prepared and two unstained 3µ thick sections were distributed to 220 participants in the technical program. The assessment method used was that the minimum requirements of a H&E should be that the H&E should demonstrate effectively 1µ the nuclear detail nuclear membranes, nucleoli, chromatin and nuclei of both vesicular and hyperchromatic nuclei and be able to define both fine and coarse chromatin; and 2) the non-nuclear components such as the cytoplasm of all cell types, fine and dense connective tissue fibres, skeletal and smooth muscle and red blood cells by contrasting well and staining differentially. The assessment was quantified by assigning a mark out of 5 to all stains submitted. Microphotograph examples were given.

Intra-laboratory comparisons showed a % improvement over 4 years and then a plateau. These improved results show the role of education in this important area of staining.

The methods used and their results were: most popular haematoxylin is Harris' (127/228), regressive more common than progressive, Scott's tap water is the most popular blueing agent.

The response given to questions of internal quality control methods are: the majority of participants (132/190) perform a check daily.

The common problems encountered by participants were: over staining with haematoxylin, poor non-nuclear component contrast and contaminants.

Key conclusions The key conclusions were that the quality of H&E seen in RCPA QAP surveys has improved over the last 4 years. Education of how to assess an H&E by internal quality control measures is ongoing.

TE00-03. Gram staining method

Mullane A, Llewellyn H., A Hopkins, RCPA QAP Anatomical Pathology, Bentleight East, Australia

The aim of this exercise was to assess the Gram staining quality by participants in the RCPA Anatomical Pathology Quality Assurance Program. Tissue was prepared according to the method of Carson for a Gram control block. This tissue was processed and 500 sections were cut and distributed to 250 laboratories in Australia and overseas. Every 20th section was stained by the Brown-Hopps method to ensure sufficient organisms were present at all levels of the tissue.

Marking criteria were discussed with a committee of scientists and the minimum requirements of a Gram stain were set.

A review of the participants' methods was made and comparison of the efficacy of each method assessed. Brown-Hopps and Gram Twort were the most commonly used methods with satisfactory results of 88% and 58% respectively.

The effect of counter-stains on the Gram staining was evaluated. The deleterious effects on Gram negative organisms especially of some counter-stains were noted, in particular picric acid and light green.

Many staining artifacts were noted and their causes identified were possible and photomicrographs of examples were taken.

Slides were assessed by two scientists and unsatisfactory slides were reviewed by a further four members of the committee of scientists. 40% of Gram stains were found to be unsatisfactory. In view of this, participants were offered further technical advice and extra slides were distributed to 32 laboratories with mentoring provided when requested.

Controls submitted were checked for staining and appropriateness of material. Many laboratories have limited access to control material and submitted smears, agar blocks and other preparations, which did not adequately demonstrate the presence of both Gram positive and Gram negative organism.

Gram staining is a routine procedure in many histology laboratories, with room for improvement in many areas, especially in the reliable staining of Gram negative organisms. This exercise will be repeated in a future quality assurance program to assess improvements in staining techniques.

Leucodepletion - the New Zealand approach

Amanda Hayward, Anne Burnand, Ray Scott. New Zealand Blood Service, Waikato, Hamilton and Auckland*

In November 1999 the Ministry of Health announced the requirement for all blood components manufactured in New Zealand to be leucodepleted. The justification for universal leucodepletion was primarily risk reduction of theoretical vCJD transmission via blood transfusion. The target date for universal leucodepletion was set at 30th June 2001.

The New Zealand Blood Service decided as a service to develop systems for universal leucodepletion based on the approach used by the National Blood Service of England. A donor deferral program was also introduced.

Leucodepletion in New Zealand is based on two process streams, whole blood and top and bottom, with a primary and back up supplier. The Whole Blood stream involves filtration of whole blood prior to centrifugation to produce leucodepleted resuspended red cells and leucodpleted plasma. Three components are created in the initial processing of Top and Bottom packs, resuspended red cells, non-leucodepleted plasma and buffy coat. The resuspended red cells are then filtered and four Buffy Coats with one plasma are pooled by the train method to produce leucodepleted platelet pools. Seventy percent of whole blood donations are collected into the whole blood stream, 30% into top and bottom. The target for platelet supply is 60% apheresis and 40% pools from whole blood buffy coats.

Monitoring of the process is done using NWA quality analyst software and Statistical Process Control (SPC) based on the aim to leucodeplete with 95% confidence that 99%of components manufactured have a white cell count less than $5x10^6$/unit. SPC is based on 100% testing until 125 data points, or white cell counts, are obtained. From this data NWA is able to calculate a mean (cl), upper control limit (ucl) and lower control limit (lcl). Using the data and statistics produced by NWA a confidence report was then prepared to ensure the requirements of leucodepletion were met.

Validation was initially carried out at the pilot site NZBS - Waikato. When systems were defined, equipment validated and processes documented approval was sought from the regulator, MedSafe, to release components for clinical use. As Phase one validation was completed implementation and Phase 2 began. From then on, 5 components per day, per process stream are tested for white cell contamination to ensure the process stays in control i.e) within the pre determined control limits.

The Role of the Chemical Pathologist in providing interpretation and guidance of test results in the community laboratory

Dr Cam Kyle, Chemical Pathologist, Diagnostic Medlab, Auckland

The chemical pathologist has an overseeing role to ensure results provided are clinically reliable, understandable and hopefully understood by those managing the patient. This role may include:

- providing value-added information on interpretation with the report itself, or if the requesting doctor phones to discuss.
- informing requesters of the most clinically appropriate tests to follow through on abnormal results and investigate problems further
- alerting users to potential errors in interpretation, such as effects of non-thyroidal illness or drugs, errors in collection time, effects of haemolysis etc.
- in some cases arranging for follow up on potentially serious results

False positive Troponin I due to platelet interference in the AxSYM Troponin I assay.

Don Mikkelsen, Sydney Sacks, Deborah Glen, Laith Aish, Dep. of Clinical Biochemistry, Health Waikato Laboratory, Waikato Hospital, Hamilton*

Background Interference in immunoassays is well documented (1,2). Recently some attention has been drawn to false positive Troponin I results using the AxSYM Analyser (Abbott Laboratories, Abbott Park, IL) due to heterophilic antibodies (3), misalignment of bulk solution dispenser no 3 (4), rheumatoid factors (5), and fibrin (6). We report incidences of false positive Troponin I levels that are attributable to platelets present in plasma samples that are incompletely centrifuged or disturbed following centrifugation.

Methods We prepared samples of platelet rich plasma and matched platelet poor plasma from lithium heparin blood samples taken from healthy volunteers. A dilution series of platelet rich plasma was made in matched platelet poor plasma to obtain a range of platelet concentrations. Cardiac Troponin I was estimated on the resultant solutions using the Abbott AxSYM.

Results Troponin I values increased with increasing concentrations of platelets in the sample from an initial value of 0.0 μg/L to a value of 2.5 μg/L. at a platelet count of 297 x 10^9/L. The relationship approximates linearity with a threshold effect at a platelet count of 44 x 10^9/L. (Troponin I = 0.108 x platelets - 0.475)

Conclusions False positive results (> 0.4 μg/L) for cardiac Troponin I on AxSYM can occur when platelets are present in the plasma sample and could lead to misdiagnosis of myocardial injury in patients whose samples are affected. Laboratories who are processing plasma samples for cardiac Troponin I on the Abbott AxSYM must ensure that centrifugation is sufficient to exclude platelets from the sample that is to be analyzed.

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Immunophenotypic analysis of lymphomas and chronic lymphoproliferative disorders

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Current approaches in the diagnosis of the chronic lymphoproliferative disorders (LPD) and Lymphomas involve multi-parameter assessment to diagnose and classify these entities in a reproducible and clinically relevant manner.

The Revised European-American Lymphoma (REAL) Classification, produced by the Lymphoma Study Group in 1994, defined lymphoproliferative neoplasms according to a set of five properties: morphology (histology), clinical features, immunophenotype, genotype, and normal cell counterpart. The World Health Organization (WHO) Classification Scheme introduction in 1997, adopted, refined and updated the REAL Classification for lymphoid neoplasms, as part of it's comprehensive classification of all haematological malignancies.

Our laboratory performs immunophenotyping studies utilizing flow-cytometry and immunoenzyme techniques to identify and sub-classify Lymphomas and chronic LPD within the major categories currently recognized : B cell neoplasms, T cell and NK cell neoplasms, and Hodgkin's Lymphoma. Cells are obtained from a variety of sources : peripheral blood, bone marrow, fine needle aspiration of lymph nodes, tissue biopsy cell suspensions, and body fluids, and are tested using panels of monoclonal antibodies . Morphology of the cells, and in many cases clinical findings influences the choice of immunophenotypic markers used to screen for and fully identify lymphoid neoplasms, and just as importantly, differentiate them from normal or reactive lymphoid populations.

The growing selection of monoclonal antisera available and advances in flow-cytometric analysis has shown the enormous heterogeneity of the lymphoid neoplasms. Immunophenotyping plays a complementary role in providing improved classification and characterization of these disorders, to enable appropriate treatment, and facilitate a greater understanding of this diverse disease group.

East meets west under the microscope. A comparison of blood film microscopy between mid-western Nepal and the Waikato region in New Zealand

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Introduction An evaluation of blood film microscopy services was carried out in the Mid-Western region of Nepal and the Waikato region in New Zealand to define strengths and weaknesses, and thereby further enhance the quality of service provided.

Methods Questionnaires and blood films compared qualifications and experience of staff, training resources, technique in preparation, examination, reporting and referral of blood films, and participation in and assessment of the external quality assurance program utilised.

Results Evaluation of the two regions with international recommendations, revealed that while many technical aspects are similar, training and resources are limited in the Mid-Western region. In the Waikato staffing is higher, specialized staff are easily accessible and abnormal films are regularly examined. Examination of blood films between the regions revealed a lack of accuracy in the Mid-Western region, while external quality assurance (EQA) programs with an emphasis on education are of great benefit to laboratories in maintaining competence.

Conclusions The ability to utilize technology and computers is very limited, and increasing staff numbers is difficult to achieve in Nepal in the immediate future. However, improving resources and training of staff can be achieved through regular refresher training, morphology workshops, distribution of abnormal films with educational supplements, and supply of blood atlases and sets of teaching slides. In New Zealand it is important for small satellite laboratories to maintain quality through regular participation in EQA programs and morphology workshops, and blood atlases and sets of teaching slides should be utilized frequently.

Experience with real time PCR in a diagnostic microbiology laboratory

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The introduction of real-time PCR technology in clinical microbiology laboratories offers the potential for the rapid detection and quantification of pathogens. It is possible to convert with some modifications existing PCR protocols onto the LightCycler. We have developed and evaluated a real-time PCR method suitable for use with the LightCycler system for the simultaneous amplification of the staphylococcal nuc and mec genes. The *nuc* gene encodes the thermostable nuclease (TNase) which is specific for *S. aureus*, and the *mecA* gene encodes the penicillin-binding protein 2a which is associated with staphylococcal methicillin resistance.

The multiplex LightCycler PCR assay has been used to test 527 strains of methicillin resistant and susceptible *S. aureus* and coagulase-negative staphylococci (CNS). The results obtained were compared with the conventional PCR multiplex assay and disk diffusion susceptibility testing.

The multiplex LightCycler PCR assay was able to correctly identify 274/275 MRSA, 63/63 methicillin-susceptible *S. aureus*, 97/97 methicillin-resistant CNS and 92/92 methicillin-susceptible CNS. Results were available within 1.5 h for the real-time PCR assay compared to up to 5 h for the conventional PCR assay.

The LightCycler provides the platform to perform an assay which is capable of accurately and rapidly identifying MRSA, which can be easily incorporated into a diagnostic clinical microbiology laboratory.

Pneumocystis carinii is an opportunistic pathogen that commonly causes respiratory infections in immunocompromised patients, including those infected with human immunodeficiency virus (HIV). An assay, using the LightCycler platform, was developed from the modification of an existing conventional PCR method for the detection of P. carinii. Both the conventional PCR assay and the real-time PCR assay were used to test forty three induced sputum and bronchial washing specimens. Twenty specimens (46.5%) were positive in both the conventional PCR assay and the real-time PCR assay and twenty three (53.5%) were negative in both assays. The melting points of the amplified products were determined on the LightCycler, and the temperatures ranged from 80.4(C to 83.9(C. All results were concordant between the two PCR assays for the specimens tested. Importantly, the results for the LightCycler PCR were available within two hours compared to 11 hours for the conventional PCR assay.

Evaluation of two rapid tests for the detection of methicillin resistance in Staphylococcus aureus

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Objectives Two rapid kits, the VelogeneTM Rapid MRSA Identification Assay (Alexon-Trend), a qualitative DNA probe test for the detection of the mecA-gene in *Staphylococcus aureus*, and the MRSA-Screen Test (Denka-Seiken Co. Ltd.), a slide latex agglutination test for the detection of PBP 2a in *Staphylococcus aureus* were evaluated. The evaluation included the ease of use of these assays in a routine medical microbiology laboratory and the value of the result to the clinician and patient.

Methods A total of 158 *Staphylococcus aureus* isolates were tested. Fifty-four were methicillin susceptible and 104 resistant .The tube coagulase test was used to identify the isolates as *Staphylococcus aureus*. Coagulase negative Staphylococci were not tested in this study. The oxacillin MIC of an isolate was determined by using AB BIODISK E-test strips. Methicillin resistance was defined as the presence of the mecA-gene or an oxacillin MIC >8 ug/ml. MecA-gene presence was detected by PCR on isolates with an oxacillin MIC of between and including 1-8 ug/ml.

Results All 54 methicillin susceptible strains tested negative by the MRSA Screen test and the VelogeneTM (100% specificity). Ninety-six of the 104 (92% sensitivity) MRSA isolates tested positive with the MRSA Screen. VelogeneTM tested 102 of the 104 MRSA isolates as positive (98% sensitivity).

Conclusions The VelogeneTM and the MRSA Screen are highly sensitive and specific assays and can be performed rapidly in a routine medical microbiology laboratory. MRSA Screen offers results within half an hour and is easy to perform. VelogeneTM produces the more sensitive result in 90 minutes. Both assays offer clinicians valuable results to enable them to reduce the patients personal costs and the financial cost to the taxpayer of an MRSA infection or outbreak. They are also helpful tools in the prevention of antibiotic resistance.

Streptococcus agalactiae neonatal sepsis

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Streptococcus agalactiae (group B streptococci, GBS) was first recognized as a human pathogen in 1935. Much later in the 1970s it was recognized as a cause of post-partum and neonatal infections. GBS today is still a rare cause of neonatal disease that is potentially very serious and sometimes fatal.

GBS are considered normal flora of the vaginal and gastrointestinal tracts. If a woman is colonized, then there is a 75% chance that the neonate will be colonized. However, despite this, only 1-2% of neonates colonized will be affected. This makes clinical history, laboratory results and maternal risk factors extremely important in the assessment of each case especially if preventative treatment is to be given.

A recent case study of severe GBS disease at Dunedin Hospital illustrated just how important maternal risk factors are in the absence of laboratory culture. GBS isolated from urine earlier in the pregnancy and a premature birth were the only known factors indicating a risk to the neonate.

New laboratory procedures are important. A new immunoassay method that does not require culture and is very rapid is now available in New Zealand. A selective broth medium is highly recommended for culture and can increase the isolation of GBS by 30% or more.

There are various problems associated with preventative treatment. In my opinion, by assessing clinical history, culture results and maternal risk factors, this disease is preventable. It is also essential that all approaches to managing this disease, including laboratory testing, are standardized and controlled to prevent more cases occurring in the future.

Significance of toxin(s) A and B enzyme immunoassay for detecting C.difficile in stool specimens

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Clostridium difficile associated diarrhea (CDA) is a very common nosocomial infection. This contributes significantly to patient morbidity

and mortality as well as costs of hospitalization. Exposure to antibiotics is thought to allow proliferation of toxigenic *C.difficile* by disrupting the normal flora. Two toxins, A and B, are associated with disease caused by *C.difficile*. These toxins are immunochemically and biologically distinct. Toxin A has been described as enterotoxin and causes an increase in intestinal permeability with subsequent enteric fluid accumulation and diarrhea. Toxin B is a potent cytotoxin, which causes rounding of cells. Testing for Toxin A or Toxin B alone will result in a highly false-negative rate and frequent misdiagnosis. Laboratories that perform both toxins A and B EIA identify significantly more infections.

The purpose of this study was to emphasize the significance of toxin A and B ELA kit as against toxin A ELA kit along which is used by most clinical laboratories for identifying *C.difficile* positive specimens. Two trials were conducted. First a trial of 91 frozen faecal samples were tested, as a result 36 were positive for *C.difficile* toxin(s). Of those, 12 were toxin A positive, and 24 were toxin B positive. Another trial was done on 142 fresh diarrhea stool specimens examined for toxin A (ProspecT, Alexon, Trend) EIA and toxin A and B (Premier, Meridian Diagnostics). Seventy-four were positive for *C.difficile* toxin(s) A and B, of those 20 were positive using EIA toxin A alone.

In conclusion, this study highlights that more effective diagnostic methods should detect both toxin(s) A and B. This should be accompanied by a warning that a significant percentage of *C.difficile* infections will be undetected by a single toxin assay. Given such a warning, physicians might routinely order assays for both toxin(s) A and B or order screening for second toxin if results for the first is negative to address the problem of under diagnosis.

New testing platforms

Lucinda Masson. Acting Team Leader Donor Accreditation, New Zealand Blood Service

The New Zealand Blood Service has made a commitment to providing two accreditation centers with up-to-date testing platforms. This philosophy provides a basis for producing a standardized system and as a consequence a quality product.

The new testing platforms to be discussed include the Abbott Prism and the Qasar III system. The Abbott Prism system is an automated infectious serology analyzer that utilizes a chemiluminescent immunoassay technique to detect anti-HIV, anti-HCV and Hepatitis B surface antigen.

The Qasar III system combines robotic liquid sample handling with image analysis of agglutination reactions. The Qasar III system primarily performs the ABO/Rhesus grouping and antibody screening but has been configured to perform other software functions.

The Abbott Prism and the Qasar III systems allow the New Zealand Blood Services accreditation departments to provide accurate and timely results.

New and future scourges of blood transfusion

Dr Peter Flanagan. Medical Director, New Zealand Blood Service

The blood available for transfusion today is safer than it has ever been. Despite this public concern over the risks of transfusion remain. Concerted efforts to reduce the often already small risks associated with virus transmission continue. Increased awareness of risks associated with other transmissible agents such as bacteria and prions is also evident.

A number of specific developments are of particular note.

Firstly the appropriateness of extending current NAT testing systems to detect other viruses. Hepatitis B virus and parvovirus B 19 are possible candidates.

Secondly the use of inactivation technologies applied to blood components. The Baxter/Cerus S 59 aminopsoaralen system has recently completed phase III clinical trials. This system utilises a light dependent psoralen to inactivate viruses in plasma and platelet components. The system has other benefits including an ability to destroy bacteria and protozoa present in donated blood. Gambro BCT has also developed and patented a riboflavin based system that is effective in both plasma and red cell components. The ability to effectively sterilize blood components is clearly attractive, care will however need to be taken to ensure that new risks are not introduced as these new technologies move closer to routine clinical use.

Increased concern over the, as yet, theoretical risk of transmission of vCJD by blood and blood products have led the American Red Cross (ARC) to intensify donor exclusion criteria to include donors who have been resident in Europe. The US FDA is also developing draft guidance in this area. Surveys of New Zealand donor travel patterns indicate that over 5% of current donors would be excluded in NZBS were to adopt the approach proposed by the ARC. Theoretical risks will need to be carefully balanced against the risk of non-availability of blood. Significant developments have also occurred in the development of diagnostic and screening tests for the infectious agent.

The opportunities to further improve overall blood safety continue to increase. Mechanisms will need to be developed to consider these in the context of other developments in Healthcare.

Interpreting thyroid function tests: clinical, sub-clinical, non-clinical and pregnancy

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TSH-centered strategies have simplified algorithms for the thyroid screening, diagnosis & monitoring. Sensitive TSH assays give undetectable values in ~ 99% of patients with clinical hyperthyroidism. Low but detectable TSH values generally exclude hyperthyroidism. Exceptions are the coexistence of heterophile antibodies, TSH secreting pituitary adenoma or the thyroid hormone resistance syndrome.

Abnormal TSH values remain a reliable guide to true thyroid dysfunction in patients taking amiodarone, lithium, carbamazepine or phenytoin. Sub-clinical or mild hyperthyroidism, (undetectable TSH, normal FT4 & FT3), can be monitored in the well young: investigation & therapy of thyroid autonomy may prevent atrial fibrillation in older patients. Raised FT3 values are very specific for true hyperthyroidism, occur early in relapse & persist during thionamide treatment.

Persistent sub-clinical or mild hypothyroidism, (raised TSH, normal FT4) is commonly caused by autoimmune thyroiditis with positive autoantibodies, and can simply be treated to restore a healthy normal TSH (0.4 - 2.0 mU/l). In hypopituitarism, 'low FT4 + normal TSH' is the usual finding.

Optimal fetal brain development is dependent on an adequate maternal thyroxine supply until the fetal thyroid takes over from mid-gestation. Observational studies suggest that even a low normal maternal free T4 value < 10th centile (< 10.5 pmol/l) in early pregnancy is insufficient. Antenatal screening and T4 supplementation above this level is prudent now.

Serum thyroglobulin is a useful tumour marker in treated thyroid carcinoma patients. Undetectable values obviate the need for regular diagnostic scintiscans.

Further reading: Lancet 2001; 357: 619-24.

MRD detection in CML using Real-Time quantitative PCR

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The molecular method of reverse transcription polymerase chain reaction (RT-PCR) has been used for a number of years to detect the RNA transcript of the BCR-ABL oncogene in cases of chronic myeloid leukaemia (CML). RT-PCR can detect residual leukaemic cells after treatment at the level of one cell in a background of 10^5-10^6 normal cells. While this sensitivity is useful for detection of MRD the assay is limited because it cannot quantify the amount of transcript remaining. Monitoring for a rising level of BCR-ABL transcript is more useful in predicting subsequent overt relapse.

Initially the method of choice for quantification of BCR-ABL was by competitive PCR assays. However, these assays have practical limitations and are time-consuming. The introduction of Real-Time PCR assays provide a rapid and reliable method of quantification which is more suited for use in a routine laboratory.

The Taqman Real-Time PCR technology for quantitative detection of BCR-ABL transcripts is currently being trialed in this laboratory. The assay utilises the 5' nuclease activity of Taq DNA polymerase to cleave BCR-ABL specific fluorogenic probes during PCR. Real time monitoring of the increase in fluorescence indicates the accumulation of PCR products and by kinetic analysis, the initial copy number of the BCR-ABL transcript. Initial results indicate that the Taqman system for Real-Time PCR may be a very useful diagnostic tool to detect molecular relapse in CML patients, and other haematological malignancies in which an abnormal transcript is produced.

The PML protein localization assay - for rapid detection of t(15;17) in patients with Acute Promyelocytic Leukaemia.

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Acute Promyelocytic leukaemia is characterized morphologically by the presence of promyelocytes and by a specific chromosomal translocation t(15;17). This translocation brings together the PML gene from chromosome 15 and the retinoic acid receptor-_ (RARA) gene from chromosome 17. With the resulting hybrid gene producing a chimeric PML-RAR protein. The disruption of the normal localization of the PML protein forms the basis of the PML protein localization assay.

The assay, a slide based test where unconjugated anti-PML antisera and a fluorescent label are added to cells to visualize the PML protein, was initially evaluated as a retrospective study involving bone marrow samples from patients with APML, microgranular variant APML and non M3 AML. All patients tested were correctly identified as either having the translocation, or not, as confirmed by cytogenetics and RT-PCR.

The slide method is currently performed on a routine basis in our laboratory and we are now evaluating whether this test would be suitable for flow cytometry. The method is the same as the slide technique but inside a test tube rather than on the slide.

Initial studies on APML patients have shown a shift in fluorescence suggesting that this may be a suitable method for detection. Control samples from non APML leukaemia were also tested and these showed no shift.

Treatment of APML patients with ATRA has previously waited for the results of cytogenetics or molecular analysis, with a minimum turnaround of 24 hours. The PML Protein localization assay has now taken over this role with far quicker results, in under 3 hours. The slide based test has proven to be rapid, sensitive and accurate and we are now investigating the use of flowcytometry more fully.

Detection of minimal residual disease in acute leukaemia

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Acute leukaemia is a clonal disorder featuring uncontrolled proliferation of malignant, immature blast cells. It is a disease which proves rapidly fatal without appropriate treatment. Although treatment protocols have come a long way in the last 10-15 years, and complete haematological remission is obtained in the majority of cases, relapse is common, with approximately 20% of childhood acute lymphoblastic leukaemia (ALL) , and 50-70% of adults and children with acute myeloid leukaemia (AML) experiencing relapse.This suggests the persistence of residual leukaemic blast cells.

Detection of minimal residual disease (MRD) is the finding of very small numbers of malignant blast cells in the bone marrow after treatment. Potential therapeutic applications of MRD monitoring are the measurement of early response to treatment, and identification of patients with high risk of relapse, leading to modification of therapy or early intervention with more intensive therapy.

A number of useful diagnostic tools are now available to assess and quantify MRD. These include conventional morphological and cytogenetic analysis of bone marrow, fluorescence-in-situ hybridisation (FISH) techniques which utilize chromosome and gene specific probes, and PCR based techniques to detect the molecular targets of chromosomal abnormalities, and leukaemia associated rearrangements of the immunoglobulin and TCR genes. There are also a number of multi-parameter flow-cytometric approaches to identify and monitor the level of malignant blast cells. When used in combination these techniques may provide timely and sensitive monitoring of treatment and assist in the challenge to avert relapse.

Nosocomial infection in New Zealand

Dr Arthur Morris. Clinical Microbiologist, Auckland Healthcare and Diagnostic Medlab, Auckland

Infection control programs are one of the most cost-effective interventions in the healthcare system. Hospital acquired (nosocomial) infections cause significant morbidity, occasional mortality, prolong hospital stay, and are a fiscal and resource drain on hospitals. The cost of nosocomial infection in New Zealand public hospitals is estimated to be more than NZ$40 million a year.

Point prevalence surveillance of nosocomial infection has been undertaken in Auckland Healthcare Hospitals every six months for the past 5 years. The most common types of infections are: lower respiratory tract 19%; urinary tract 17%; surgical site infection 17%; blood stream 15%; and skin and soft tissue. The most commonly identified pathogens are: *S. aureus* 17%; *E. coli* 10%; other *Enterobacteriaceae* 9%; *P. aeruginosa* 5%; *streptococci* 4%; *C. difficile* 4%; and *C.albicans* 3%.

Risk factors for nosocomial are common: a third of patients have had an operation; a third have an intravascular line in place; 11% have urinary catheters; and 13% have other invasive devices in place, e.g. surgical drains, nephrostomy tubes.

The clinical microbiology laboratory should play an active role in the diagnosis, monitoring, and reporting of nosocomial infection. The first suspicion of an outbreak due to a particular organism often occurs within the laboratory. Early notification of the presence of transmissible multi-resistant organisms from the clinical microbiology laboratory is capable of limiting the spread of infection.

Pulsed-Field Gel Electrophoresis: the use of this technique in the Enteric Reference Laboratory at ESR

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Pulsed-Field Gel Electrophoresis (PFGE) is a molecular technique used to compare isolates that cannot be distinguished by other methods. The technique involves extracting bacterial DNA and treating with restriction enzymes, which cleave the genome into fragments of varying sizes. The large fragments thus generated require electric current applied in two different directions to move them through an agarose gel matrix. Isolates treated in this way provide a genetic fingerprint. In an outbreak situation, PFGE can be used to distinguish between isolates from the outbreak and isolates resulting from sporadic infection. An isolate can be defined as "definitely", "probably", "possibly" or "not" part of an outbreak depending on the PFGE pattern obtained and the number of differences between it and the outbreak strain.

Recent isolates characterized by PFGE at ESR include *Salmonella brandenburg, Salmonella typhimurium* phage type 160 (STM 160), *Shigella sonnei* Biotype a, and *Escherichia coli* O157. We have been able to recognise specific clones of *S. Brandenburg* and STM160, and to trace an outbreak of *Shigella sonnei* Biotype a. Two cases of infection with *E coli* O157 were linked to water isolates, and one to a bovine source using PFGE.

Automation of an immunohaematology laboratory

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Automation offers several advantages over traditional pre-transfusion testing protocols including standardization of tests, faster specimen processing and reporting, objective and uniform reading of haemagglutination endpoints, interpretation of serological reaction patterns and decreased hands-on operator time. Transfusion services have often lagged behind other diagnostic pathology laboratories in automating because until recently appropriate instrumentation was not available. However, the shift from tube to column agglutination techniques has now allowed fully automated instruments to be developed.

The AutoVue™ is a fully automated walk-away random access instrument that uses column agglutination technology to perform a broad range of pre-transfusion tests including ABO/Rh groups, antibody screens, direct antiglobulin tests, crossmatching and Rh phenotypes.

This instrument was recently introduced into our service. 90-95% of our routine pre-transfusion testing has been now been completely automated. Following automation, specimen processing and reporting times have fallen by 30-40%. Actual hands-on technical time to process specimens using the AutoVue™ compared to tube techniques has decreased by 79%. Increased efficiency has been achieved through attrition as positions became available with minimal impact on staff morale even though workload has increased by 36%. At the same time productivity has increased by 31% with maintenance of test result reliability without a corresponding increase in error rate or testing discrepancies.

Introduction of automated pre-transfusion testing has seen significant improvements in specimen processing times, productivity and efficiency along with improved accuracy and test reliability within our Transfusion Service.

Stress, emotions and immunity

Dr Roger Booth. Department of Molecular Medicine, Faculty of Medical and Health Sciences, University of Auckland

Over the past 10 years research has revealed a variety of connections between the immune system and the nervous system, involving cytokines, neuroendocrine hormones and autonomic nerves. These provide mechanistic pathways through with immune activity affects the brain and nervous system such that our thoughts, feelings and behaviors are altered during illness. Because these pathways provide bi-directional communication routes between immune and nervous systems, they also provide mechanisms whereby our thoughts, feelings and behaviors can modulate immune activity. One of the most-studied aspects of these neuroimmune connections is the relationship between stress, emotions and immunity.

Data management on point of care analysers.

Rose-Marie Daniel. Radiometer Pacific

Management of point of care testing (POCT) equipment. Testing equipment operated remotely from an accredited laboratory shall be subject to the same maintenance, calibration and QC criteria applied to equipment located within an accredited laboratory.

Training of staff. All staff using POCT equipment shall have successfully completed a comprehensive training program in the use of the equipment. Training records for non-laboratory staff shall be kept and should be reviewed regularly.

Responsibility for POCT results. The accredited laboratory within an institution or laboratory service shall accept responsibility for the management of POCT equipment associated with that institution or service, including the training of staff using the equipment.

IONman or IRONman? Response to a fluid load in athletes with a history of exercise associated hyponatremia

DB Speedy *FACSP, FACSM; TD Noakes MBChB, MD, FACSM; T Boswell; JMD Thompson PhD; N Rehrer BA, MSc, PhD; DR Boswell MBChB, PhD, FRACP*

Purpose To determine whether athletes who had previously developed hyponatremia during an ultradistance triathlon show an impaired ability to excrete a large fluid load compared to athletes who had completed the same race without developing hyponatremia.

Methods Six athletes who had developed hyponatremia ([Na^+]<135mmol/L) in the 1997 Ironman triathlon (study cases) were compared with six athletes who completed the same race without hyponatremia (controls). All participants consumed 3.4L of water over 2 hours at rest. Weight, urine output, urine electrolytes, serum [Na^+], hemoglobin and hematocrit were measured every 30 minutes. Changes in plasma volume and residual fluid volume in the gut were estimated from these data.

Results There were no significant differences between cases and controls in any parameters measured. Maximal rates of urine production (± SD) (1043 ± 331 mL/h for cases, 878 ± 168 mL/h for controls) were substantially behind the rate of fluid intake (1500mL/h). Consequent to fluid retention, serum [Na^+] fell progressively in both groups. Five cases and four controls developed hyponatremia. There was an inverse correlation between change in body weight and change in [Na^+] (r=-0.67). Estimated changes in the intra- and extra-cellular fluid volumes could account for all the retained fluid and there was little evidence for fluid accumulation in the bowel.

Conclusion When evaluated at rest, there does not appear to be any unique pathophysiological characteristic which explains why some athletes develop hyponatremia in response to fluid overload during prolonged exercise. Rather hyponatremia was induced with equal effect in both cases and controls, consequent to progressive fluid overload of all the body fluid compartments and without evidence for fluid retention in the small bowel.

Dr Dale Speedy is a sports physician in private practice in Auckland. He is a research fellow at the University of Auckland completing a doctoral thesis in medical complications of ultra-distance exercise. Dr Speedy is also Editor of the New Zealand Journal of Sports Medicine.

Childhood Acute Lymphoblastic Leukaemia

Dr Lochie Teague. Auckland Hospital, Auckland

Acute Lymphoblastic Leukaemia (ALL) is the most common form of cancer in childhood, accounting for 30-35% of cases of malignancy equating to 40-50 children per year in New Zealand.

As our understanding of the biology of cancer has increased enormously over the last few decades, so too have the treatment protocols. By applying both simple as well as complex laboratory tests, we are better able to adapt treatment according to an individual's "risk".

Diagnosis of ALL is accurate for 80% of cases with standard morphology and cytochemistry. This accuracy is further improved by 10% with immunophenotyping and now karyotyping, FISH and PCR analyses by a further 5-10%.

Children with standard risk ALL as defined by NCI criteria, 1 through 9 years of age, WBC <50 x 10^9/L comprise 60% of all cases of paediatric ALL. Expected long-term survival for this group is approximately 80-85%. Currently all children in New Zealand are treated on the ANZCCSG Study VII protocol but it is anticipated that within the next 3-6 months, the CCG 1991 protocol will be open for patient entry. This is a huge multi-center collaborative study. In addition to testing therapy, it will aim to determine if measurement of peripheral lymphocyte count and/or blast clearance during the first week of therapy can act as a surrogate for early bone marrow relapse. Further examination of the biological factors TEL-AML, fusion transcript, trisomies of chromosomes 5, 10 and 17, will also be undertaken. To answer questions with any statistical certainly, clearly large numbers of patients are required given the overall very good prognosis. By collaboration with CCG and we will be able to contribute to the 2000+ patients required each year to answer such questions.

High risk patients require very intensive regimes and in a very small minority of patients Stem Cell Transplantation is required as part of that initial treatment. In childhood ALL, Stem Cell Transplantation only has a very limited role, however, it is generally required for patients who have marrow relapse.

Molecular mechanisms of polycythaemia

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The absolute polycythaemias are a heterogeneous group of disorders. Historically these have been divided into: primary (chiefly polycythaemia rubra vera - PV), secondary (where excessive erythroid proliferation is driven by a discernible cause), and a third group where no cause is identifiable - the idiopathic erythrocytoses.

The mechanisms responsible for some of the polycythaemias have not been elucidated, and are thus unavailable as a precise diagnostic tool. Recently, a number of cellular and molecular abnormalities in PV have been identified. Platelets from such patients appear to have a dramatic reduction or complete absence of the thrombopoietin receptor. Also, the cDNA for PRV-1, a novel haematopoietic cell surface receptor over-expressed in PV, has recently been cloned. Assays for these two defects have been designed, and may be more specific in establishing the diagnosis than current criteria.

A recently reported form of polycythaemia detected in the Chuvash population of the Russian Federation is thought to be due to an abnormality in the hypoxia sensing-pathway, involved in the regulation of EPO gene expression.

It is hoped that the definition of underlying cellular and molecular defects will improve the diagnosis of polycythaemia and lead to a clinically relevant reclassification.

Attacking New Zealand's most urgent infectious disease issue: is a solution in sight?

Diana Lennon, Diana Martin, on behalf of the Meningococcal Management Team (Diana Lennon,, Principal Investigator; Jane O'Hallahan, Ministry of Health; Philipp Oster, Chiron Vaccines; [with advisors: Sue Crengle, NHC; Diana Martin, ESR; Teuila Percival, South Auckland Health; Stewart Reid, General Practitioner, Lower Hutt; Joanna Stewart, University of Auckland]

New Zealand is in the 11th year of a large and wide spread epidemic of meningococcal disease (2000: 13.3/100,000) increasingly dominated by a single serosubtype (P1.4). To Dec 31, 2000, there have been 3547 cases and 158 deaths. Case numbers in 2001 have been above average.

Quadrivalent meningococcal polysaccharide vaccine is efficacious for serogroup A,C, W135 & Y disease. Serogroup B polysaccharide antigen is poorly immunogenic and the generation of antibodies to serogroup B polysaccharide antigen is of concern because this antigen is present in human neonatal neural tissue. Thus strategies for serogroup B vaccine development have focussed on outer membrane protein (OMP) antigens.

The World Health Organization reaffirmed New Zealand's approach to seek an outer membrane protein serosubtype - specific ('designer') vaccine and in 1998 invited 4 manufacturers to submit an capability and capacity, suggested as a result competing phase II (immunogenicity) studies.

From late 1999, the persistence of the epidemic and the increasing confidence in serum bactericidal antibodies evolved to an approach designed for more urgent epidemic control. This was supported by the UK approach to meningococcal C disease control (rate ~ 3 / 100,000) where vaccines were licensed based on immunogenicity and safety data alone1.

The serum bactericidal activity (SBA) assay has become the most important serologic correlate for meningococcal vaccine efficacy best shown by gp A&C vaccine studies[2-4]. The SBA has also become the primary serologic assay used to assess protective immunity stimulated by serogroup B meningococcal vaccine candidates,[5-11]. Recent evidence suggests that class 1 outer-membrane protein (OMP) (encoded by the *porA* gene) plays a major role in the SBA immune response following meningococcal carriage[13], invasive disease[14] and immunisation with a serogroup B OMP meningococcal vaccine in adults, infants and children.[10-12,15,16] In addition studies using altered Norwegian gp B vaccine strains have confirmed that SBA induced by the OMP vaccine was mediated primarily through the specific class 1 OMP.[17]

Thus New Zealand's likely approach[18] through a consortium of the Ministry of Health, Chiron Vaccines and a research team lead from the University of Auckland is to advance to vaccine licensure and epidemic control with vaccine effectiveness[19] evaluation, following safety and immunogenicity trials in adults.

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The Impact that vCJD will have in New Zealand

Dr Peter Flanagan. Medical Director, New Zealand Blood Service

Variant Creuzfeldt-Jakob Disease (vCJD) is the human form of bovine spongiform encephalopathy. The disease in humans arises from ingestion of contaminated beef and beef products. The disease was first diagnosed in 1996. To date there have been approximately 100 cases diagnosed in the UK. 3 cases have been confirmed in Europe and recently a case was confirmed in Hong Kong. There is considerable doubt as to the likely future scale of the disease.

Initially considered as a 'British disease' it is now clear that the disease has a capacity to impact more widely. A number of factors will contribute to this. These include international travel patterns, historic exports of beef and beef products from the UK and the export of meat and bone meal from the UK. The recent increase in reports of cases of BSE in European countries also contributes to the concern.

The European Union has recently published a Geographic classification on the BSE risk (GBR). This classification aims to provide an objective assessment of the likelihood that cases of BSE will occur and also the ability of local surveillance systems to detect them. New Zealand is one of only 5 countries with a GBR status of 1, the lowest risk.

The risk of BSE being diagnosed in New Zealand is low. The same is not necessarily true of vCJD. Precautionary measures have already been introduced to protect both the food supply and also to reduce the risk of transmission by blood and blood products. The MoH has recently announced an extension of the ban on importation of beef products. Initially relating to products manufactured in the UK this has now been extended to include 15 European countries. Universal leucodepletion of blood components and a UK donor deferral have been introduced to protect the blood supply. Consideration is now being given to extension donor deferral to include donors who have lived in Europe.

A review of the contributions by New Zealand to blood transfusion activities in developing countries

Dr. D. Graeme Woodfield. Clinical Associate Professor in Transfusion Medicine, Division of Molecular Medicine, Auckland University

There is a great need in many developing countries to improve health services by creating National blood services that are efficient and safe. Unfortunately, it is common for financing of blood services to be given a low priority in such countries. Blood transfusion services are often developed as an appendage to individual laboratories without clear direction and lacking a national coordinated basis.

NZ is fortunate in having one of the best transfusion services in the world. Over the years, NZ has been able to share aspects of this expertise with a variety of Asian and Pacific countries and has had a significant influence in countries such as Nepal, Thailand, Papua New Guinea and Viet Nam as well as a variety of the Polynesian countries. NZ has also significantly contributed to many Red Cross Society and World Health Organization symposia and seminars and has been well represented at other relevant Conferences.

Aid in blood transfusion to developing countries is a slow process but can create long -term good relationships between peoples of different cultures. Foreign aid in this special area of medicine is always well received by the recipient country and can have a very marked influence on the development of their health services. It is one area in which NZ can really contribute to improvement of health services at relatively low cost and further assistance should be encouraged.

Clozaril® (clozapine) ~ why all the blood tests?

Deborah Steele, BMLS. Clozaril® Clinical Support, NOVARTIS NZ Ltd

Clozaril® is an effective anti-psychotic for treatment resistant schizophrenia giving many patients a much improved life-style. One of the side effects of Clozaril® is the risk of developing neutropenia (defined as neutrophil count < 1.5 x 10^9/L) and/or agranulocytosis (neutrophil count < 0.5 x 10^9/L)

Cause of clozapine-induced neutropenia is unknown but may be due to a direct toxic effect on the bone marrow but more likely to is immune related. Because of this Med. Safe requires that all patients receiving Clozaril® treatment must have regular full blood counts (FBC). Clozaril® is the only drug to have such a monitoring regime.

Clozaril® cannot be dispensed by pharmacists without first checking that the WBC and neutrophil counts are satisfactory. Statistics have shown that Clozaril induced neutropenia is most likely to occur in the first 18 weeks of Clozaril® therapy. Because of this patients undergo weekly FBC testing for the first 18 weeks before changing to four weekly testing for as long as they continue on clozapine. Reference guidelines defining "safe" limits of parameters for neutrophil and WBC counts are used for monitoring.

The rates of neutropenia and agranulocytosis have reduced since 1995.

Year	1995 (April)	2001(July)
Agranulocytosis	1.15%	0.76%
Neutropenia	2.02%	1.50%

Clozapine serum/plasma levels are not as important and do not need to be performed regularly. They may be used to monitor compliance or assess toxicity. There are several agents, which interact with serum levels.

All patients receiving Clozaril® are registered with NOVARTIS NZ Ltd. All patients' WBC and Neutrophil counts are monitored through the Novartis database. Results are supplied directly from laboratories throughout NZ using Healthlink (electronic mail). Mental Health Workers, psychiatrists and pharmacists have access to this database (CareLink) via a private server. Information on CareLink is viewed as web pages. CareLink is soon to be installed on the Hospital Intranet since its security approval by the Health Intranet governance body.

The aims of this service are to minimize the risk of patients receiving Clozaril® developing an agranulocytosis or neutropenia and enhance patient safety.

Fresh frozen plasma: a three month prospective audit

HA Blacklock, MJ Dewse S, Jackson. Middlemore Hospital, South Auckland Health

Fresh frozen plasma (FFP) is a valuable resource. Its availability as a source of coagulation factors has led to increased use over recent decades. In common with all blood products, FFP carries a potential viral risk. Good clinical practice would therefore avoid unjustified use of this product.

Although international guidelines have been developed for the use of FFP, audit in other centers has revealed wide variation in the rationale for the use of this product. It was therefore decided to review clinical use of this product at Middlemore Hospital, which is a 750 bed tertiary referral hospital in South Auckland, New Zealand.

This audit examined criteria for use (compared against local printed guidelines), volumes transfused and product waste. Data was collected on patients aged between 1 day and 95 years. Indications for the orders of FFP fell into three main groups: bleeding and/or coagulopathy, reversal of anticoagulation prior to invasive procedure or reversal of over-anticoagulation. Smaller numbers of patients were septic or had impaired liver or renal function. Results of pre and post transfusion INR (international normalized ratio) and APTT (activated partial thromboplastin time) were examined as was the use of vitamin K.

Although 97% of the transfusion events monitored were justified in terms of the local consensus guidelines, in some cases there could be some debate about whether treatment with FFP was best clinical management. By the nature of donation all blood products are precious and costly to process. They should be used only in specific situations when this is the optimal treatment for the individuals concerned. This paper therefore examines how well this is being achieved in respect of FFP.

Editor's note. See the Editorial in this issue of the Journal.

News from the PPTC

It has been a busy 12 months or so at the Pacific Paramedical Training Centre which is based at Wellington Hospital. The following are extracts from the Annual Report presented to the Centre's AGM earlier in the year by the Chairman, Dr Ron Mackenzie.

Retirement of Mike Lynch

Last year [2000] saw the retirement of Mike Lynch after 15 years as Tutor Co-ordinator of the Centre. To mark the occasion a farewell luncheon was held on March 3 at the Centre when many friends and colleagues gathered to pay tribute to Mike's outstanding contribution to the PPTC and health laboratory services of the Pacific Island Region. In addition to the sentiments expressed at the luncheon, many expressions of thanks and good wishes were sent to Mike from laboratories throughout the Pacific Islands. This occasion reflected the high esteem in which Mike is held by both colleagues and former students. On the occasion of this Annual Report the Management Committee of the PPTC express thanks and wish Mike and Ngaire a long and happy retirement.

The Management Committee was pleased to announce the appointment of John Elliot to Mike's position. John was formerly a Senior Medical Laboratory Scientist with Capital Coast Health, has had a long association with the PPTC and working experience in developing countries of the Pacific and Asia.

Training courses

Two training courses were held at the Centre during 2000. The first was a Quality Assurance Course held 24 July to 18 August. This was attended by 7 senior technologists and focused on quality systems as they apply to medical laboratories and the general principles of laboratory management.

The second course on Blood Bank Technology was held from 4 September to 27 October and there were 5 participants.

In addition work experience attachments were arranged for technicians from Fiji and Federated States of Micronesia in Medical Cytology and Haematology.

Laboratory quality systems

A project to introduce Laboratory Quality Systems (LQS) into the operation of the Laboratory at Tupua Tamasese Meole Hospital, Samoa and the Laboratory at Vaiola Hospital, Tonga was commenced during 1998 and is continuing. They are funded through a grant from the Ministry of Foreign Affairs and Trade.

These programmes are an effective means of improving laboratory quality and it is intended that LQS Programmes be extended throughout the Pacific Island Health Laboratory services. The Centre has proposals with the New Zealand Government ODA Programme to establish the programme in the Laboratory Services of Vanuatu, Solomon Islands and Niue.

The primary purpose of the programme is to introduce the principles of quality systems into these laboratories but it also has the longer-term goal of preparing them for accreditation.

Regional external quality assurance program

Twenty-one laboratories continued to participate in this program during 2000 and of these 19 are National Laboratories supported by WHO. The laboratories at Lae (PNG) and Qui Nhon Provincial Hospital (Vietnam) are sponsored by private arrangement. In response to WHO's request that the Centre increase the number of surveys sent to the participants, during 2000 the Centre increased the samples in microbiology, haematology and immunohaematology to three each per year.

The overall participation rate in the QA Programme for 2000 was 62% which is an 11% drop from 1999. Only 4 (19%) laboratories completed all surveys and 8 (38%) completed 50% or less. However, the results from the surveys returned continued to show an improvement in quality.

The PPTC feels that the complexity of the samples is appropriate for the level of instrumentation and expertise of laboratory staff working in the Pacific Region. The REQAP co-ordinator is in constant communications with the laboratories of the region and he and the other co-ordinators know the laboratories, their staff and laboratory equipment used.

With the increased number of surveys sent out in 2000 the costs related to the organisation of the REQAP have increased to NZ$1,350 per participant.

Other activities during the year:

- **March:** John Elliot attended the inaugural meeting of the Pacific Public Health Surveillance Network [PPHSN], Public Health Laboratory Network held at the SPC Center in Noumea.

- **March:** The Chairman, Dr Ron Mackenzie, was invited to attend and contribute to the Professor Ken Newell Colloquium held at the International Health Division, Liverpool School of Tropical Medicine.

- **June:** Michael Lynch was appointed a WHO Short Term Consultant to Pohnpei, FSM to assist in the training of laboratory staff during the cholera outbreak in that State.

- **October:** John Elliot visited the Laboratory, Vaiola Hospital, Tonga to conduct the next phase of the LQS Project.
  Following this, a visit was made to the CWM Hospital, Suva, Fiji to meet with staff and introduce the LQS Programme concept. A visit was also made to the Fiji School of Medicine to meet with Rajendra Singh, Head of the School of Medical Laboratory Technology and a courtesy call was made to the WHO Regional Office and the SPC Vector Borne Diseases Project Office, Mataika House, Tamavua Hospital Complex.

Acknowledgements

The Pacific Paramedical Training Centre is indebted to a number of organisations and individuals for ongoing support and encouragement. To the following the PPTC extend sincere thanks for generous assistance:

- The New Zealand Ministry of Foreign Affairs and Trade
- The New Zealand Ministry of Health
- New Zealand Blood Service
- Capital Coast Health
- Department of Laboratory Services, Wellington Hospital

- New Zealand Institute of Medical Laboratory Science
- Wellington School of Medicine
- New Zealand Red Cross, Central Region
- Norman Kirk Memorial Trust
- The Royal College of Pathologists Australasia
- Australasian Association of Clinical Biochemists
- CITEC Training Education Consultancy Ltd.

Photo 1.Mike Lynch's retirement function, March 3 2000

Photo 2.Quality Assurance Course ,24 July to 18 August2000
Back row: Christine Story(PPTC), John Elliot(PPTC), Faiatea Latasi(Tuvalu), Viliami Pakalani(Tonga), Tirath Lakshman(Tutor), Philip Wakem(Tutor) Douglas Tou(Cook Islands), Raymond Seule(Vanuatu), Mike Lynch(PPTC),
Front row: Tautala Mauala (Samoa), Alfred Dofai(Solomon Islands, Parmod Kumar(Fiji), Clare Murphy(Tutor).

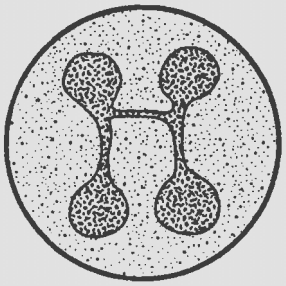
Photo 3. Blood Bank Technology Course , 4 September-27October 2000
Back Row: Tirath Lakshman(Tutor), John Elliot (PPTC), Ron Mackenzie(PPTC), Mike Lynch(PPTC), Paape Sotago(Tuvalu),
Front row: Makarita Baleinadoga(Fiji), Robert Tom(Vanuatu), Palauni Mauinata(Samoa), Peia Ben(Cook Islands).

Photo 4. Haematology and Cytology Attachments, October - December 2000

Back row: Dinesh Ram(Cytology-Fiji), John Elliot(PPTC), Robert Fauck(Tutor), Philip Wakem(Tutor)
Front row: Taina Nailevu(Cytology-Fiji), Kinisalote Sanimo (Haematology-Fiji)

Papers published in the British Journal of Biomedical Science, a publication of the Institute of Biomedical Science (UK)

Volume 58, Number 2, 2001



Journal review questions

Blood, Volume 98, No. 1, July 1 2001, p13 - 18
Review: Structure of a factor VIII C2 domain-immunoglobulin G4k Fab complex : Identification of an inhibitory antibody epitope on the surface of factor VIII.

1. What involving infused factor VIII is a complication affecting many haemophilia A patients?

2. Is factor VIII a large 2331 residue plasma glycoprotein **T/F**

3. Fill in the blanks: Haemophilia A is a _____ _____ disorder due to deleterious ____ ____ gene mutations.

4. What is the current therapy for haemophilia A patients?

5. Antibody inhibitor development has been associated with the mutations R593C and W2229C. **T/F**

6. The current working model for inactivation of factor VIII by B02C11 is
   - Antibody forms tight stable complex with FVIII as it dissociates from vWF.
   - Antibody blocks the C2 domain membrane-binding site, sequestering factor VIII
   and neutralising its effects. **T/F**

7. Initially how were Anti-factor VIII antibodies distinguished?

8. Competition with vWF for factor VIII binding was first described as the mechanism responsible for the type II kinetic pattern of many type II inhibitor antibodies. **T/F**

9. B02C11 inactivates factor VIII following a kinetic pattern intermediate between that of type 1 and type II inhibitors. **T/F**

10. Severe C2 domain missense mutations associated with haemophilia A do not appear to affect vWF binding... **T/F**

11. Recent experiments using recombinant factor VIII proteins incorporating point mutations in this region have shown that the substitutions affected the antigenicity of factor VIII against both polyclonal and monoclonal antibodies. **T/F**

Answers on page.....103

Thirteenth Annual

NICE WEEKEND

A Transfusion Science educational opportunity organised by the TSSIG

at Wairakei on 19-21 April 2002

The NICE Weekend (National Immunohaematology Continuing Education) is an educational meeting for all people working in Immunohaematology and/or blood services . As usual it will be held at the Wairakei Resort Hotel.

As always, all participants are required to participate. You must present either a poster, or an oral presentation lasting 2 to 5 minutes, on any topic related to Immunohaematology or blood transfusion. It can be a case study, a discussion, a question, a problem you want others' help with, etc. This will be followed by questions and discussion of the topic you raise. This compulsory participation makes everyone nervous (yes, even the "old hands") but it really is one of the reasons why the NICE Weekend is so successful.

The registration fee is $250, reduced to $220 for current financial members of the NZIMLS. Your registration fee entitles you to:

- two nights (Friday 19 April and Saturday 20 April) accommodation on a share twin basis
- continental breakfast, morning and afternoon teas, and lunches on Saturday and Sunday
- dinner on Saturday night. (Dress theme is HORROR!)

Transport costs will be your own responsibility.
Accommodation on other nights and other meals can usually be arranged directly with Wairakei Resort Hotel. This is also your own responsibility.
Please plan arrive at the venue on Friday evening, as we have a full programme planned.

If this is your first NICE Weekend, we will put you in contact with a "buddy" who can introduce you to everyone, explain anything you don't understand and make you feel at home.

Because participant numbers are limited to the FIFTY registrations preference will be given to NZIMLS members . We will fax your application form back to you on receipt, to let you know that your registration has been received . If you don't hear from us we have not heard from you.

If you have any questions contact
Grant Bush
ph 07 5798234
email; grantb@medbop.co.nz

NICE WEEKEND
19-21 APRIL 2002
A Transfusion Science Education Opportunity
Organised by The TSSIG

Please register me for the 2002 NICE WEEKEND

Surname:		First Name:
Address for receipt:		
Phone:	Fax:	E-mail:

Paper or Poster: (Circle) Title:

A brief abstract of your presentation MUST accompany your registration form by 19th March 2002

Is this your first N.I.C.E. Weekend:	Yes/No

Registration Fee	-$250	$
Or for NZIMLS members	-$220	
Private Room Surcharge I wish to share a room with	-$125	$ _____
Late Registration Fee (payable after 22nd March)	-$50	$
I enclose a cheque, made out to "NICE WEEKEND" for the amount of:		$

Applications received after Friday 22nd March 2002 can only be accepted if accompanied by the late registration fee. The Private Room Surcharge is payable only if you wish to have a room to yourself.

Signature:

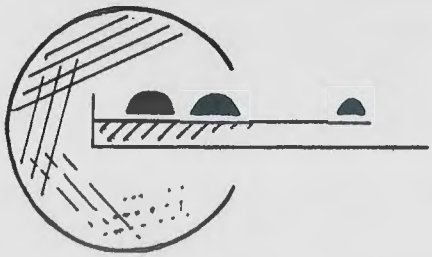
Please send form and cheque to Grant Bush, Transfusion Lab Medlab BOP P.O. Box 130, Tauranga. Note registration is limited to 50 participants and preference will be given to NZIMLS members/

Your form will be faxed back to you promptly to confirm your accepted registration.

Registration received and accepted	Date:
Number:	Signed:

Microbiology

Special Interest Group



The 2002 seminar will be held at Christchurch Hospital on Saturday May 11th. At this stage we are unsure as to whether there will be a preliminary session on the Friday. For further information contact Julie Vincent at Canterbury Health Laboratories.

This will be my last report as convenor as I will be stepping down at the end of the year. I would like to thank all those who have helped me over the last 18 months, and especially the SIG committee – Sue Earley, Jodie Cranfield, Linda Manuel, Catherine Tocker, Kay Stockman, Tina Littlejohn, and Julie Vincent.

Steve Soufflot
Convenor.

Transfusion Science

Special Interest Group

Blood transfusion reaction investigation workshop

This workshop was held prior to the NZIMLS conference in September and was very successful. This TSSIG would like to express its sincere thanks to Holly Perry and Linda Pinder for the excellent content and presentation of the workshop. The feed back we received has been very positive.

National Immunohaematology continuing education weekend.

The TSSIG will be running the National Immunohaematology Continuing Education Weekend at Wairakei next year. The dates are 19-21 April 2002. All attendees are required to present a paper or poster on any aspect of Transfusion Science. The purpose of the weekend is by definition, education ie encouragement to learn, to gain confidence at presenting and to build networks. In the past, this formula has proved a great success and all medical laboratory scientists are encouraged to attend.

Symphony of Science !!!

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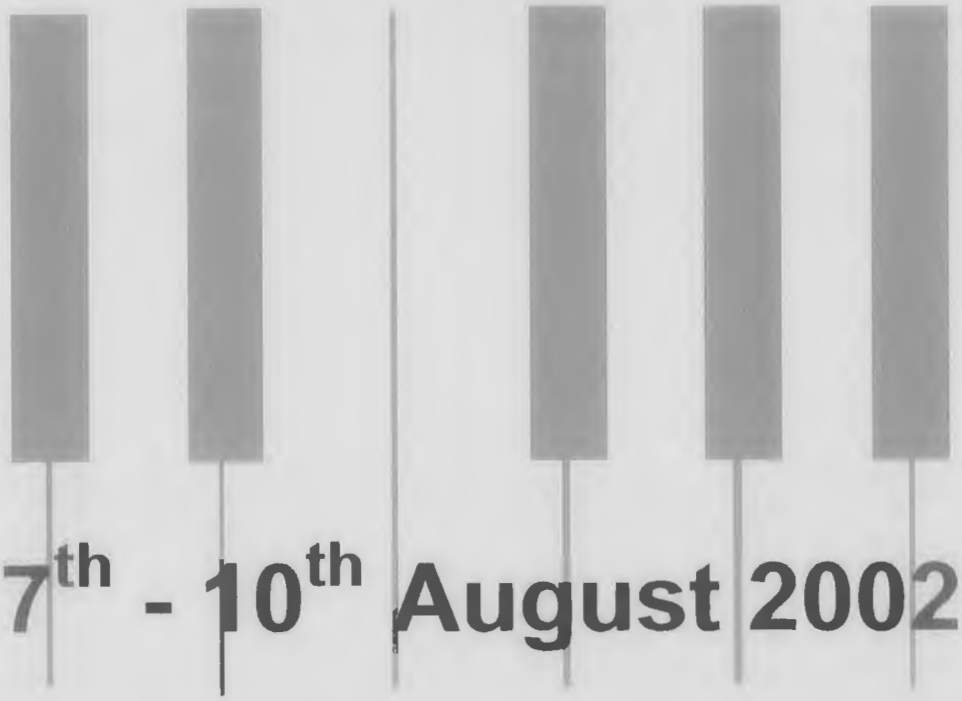

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2001 A South Auckland Odyssey Organized by South Auckland Health Laboratory.

The Annual New Zealand Institute of Medical Laboratory Science Conference entitled "2001 A South Auckland Odyssey" is over and it was pretty wonderful, lots of fun and a good and interesting time was had by all. Some 600 delegates attended the one day of workshops (308 attendees) and the three days of Conference (376 attendees). If you missed out this year there is a Symphony of Science in Wellington next year - not to be missed - and the SI Seminar to look forward to. Try to attend, they are sure to be excellent.

The terrorist attack on America on the Wednesday was shocking start for all of us. In remembrance of the horror, a minute's silence was observed at the opening ceremony on Thursday morning.

Delegates enjoyed a slightly different format this year. The daily Plenary Sessions set the mood for a relaxed and enjoyable conference. The speakers at these sessions gave us a broader perspective of the laboratory as part of the health culture. On day one we started with Ken the Weatherman with his interesting perspective on global warming. Then the conference was opened by David Clarke the CEO of the Counties Manukau District Health Board. He spoke of the Hospital's role in Primary Care and certainly gave us a lot to think over. A special thanks to Ross Anderson who expanded the TH Pullar theme and gave us some good ideas on individual motivation, vision and teamwork, or was he really just talking about the Tour de France?

By contrast the breakout sessions concentrated on the more traditional areas, we were there to experience the exchange of knowledge in our chosen fields. Even here one could observe an overlap of the disciplines and many people found themselves in sessions away from, but still relevant to, their chosen field.

This year, as well as giving us a fabulous scientific programme, Bryan Raill came up with some very special workshops. Laboratory planning was enlightening and enjoyable for all those who attended. The phlebotomy and associated pre-analytical workshops where extremely well attended, the outcome being that a phlebotomy special interest group is to be set up to discuss ideas for a national qualification. The method evaluation workshop was very well received and was a great success. Let's not forget parasitology. As a result of demand an extra workshop was included! Thanks to all those involved in the immunology, haematolgy and histology workshops.

The dinner was fabulous. Ailsa Bunker , and her social committee, went all out and created a sci-fi fantasy to dazzle us. Anyone wanting a video of the Middlemore Star Trekkies Entertainers, just let us know - weren't they grand. A special thanks to them and to Camille Ahkiau and her aerobic team. Most people came dressed as their space fantasy alter ego, and prizes for the most fantastic costume were deservedly won. Plenty of food for all but who had time to eat when the band had everyone on the dance floor until Lakeside turned out the lights at 01:00 hours.

Saturday, the last day and the mood persisted. Dr Roger Booth, a psycho-neuroimmunologist from Auckland University had everyone laughing with his little song - Megastress. Some of the best breakout sessions where saved for this day and people took advantage of it not being a work day to attend. The finale - Steve Gurney and lunch - the man is a legend.

Many thanks to the sponsors, without their financial support conference would not be the success it is. Their humour, willingness to get into themes, and of course all those lovely new analysers and ideas kept everyone buzzing in the breaks. Plus we loved the ice creams and the coffee.

Special thanks must also go to the total commitment shown by the Middlemore laboratory staff, their willingness to carry the extra workload, and to get involved in skits "that required dedication above and beyond the call of duty". Many, many thanks to the management staff of Middlemore Laboratory and South Auckland Health for their total commitment and support.

Dade Behring Group

Dancing at conference

Mad Max M Bernard

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Answers to HSIG journal review questions

1. The development of an immune response

2. False

3. Congenital bleeding. Factor VIII

4. Current therapy involves therapeutic infusions of factor VIII.

5. True

6. True

7. Initially distinguished according to the kinetics of factor VIII inactivation.

8. True

9. True

10. False

11. True

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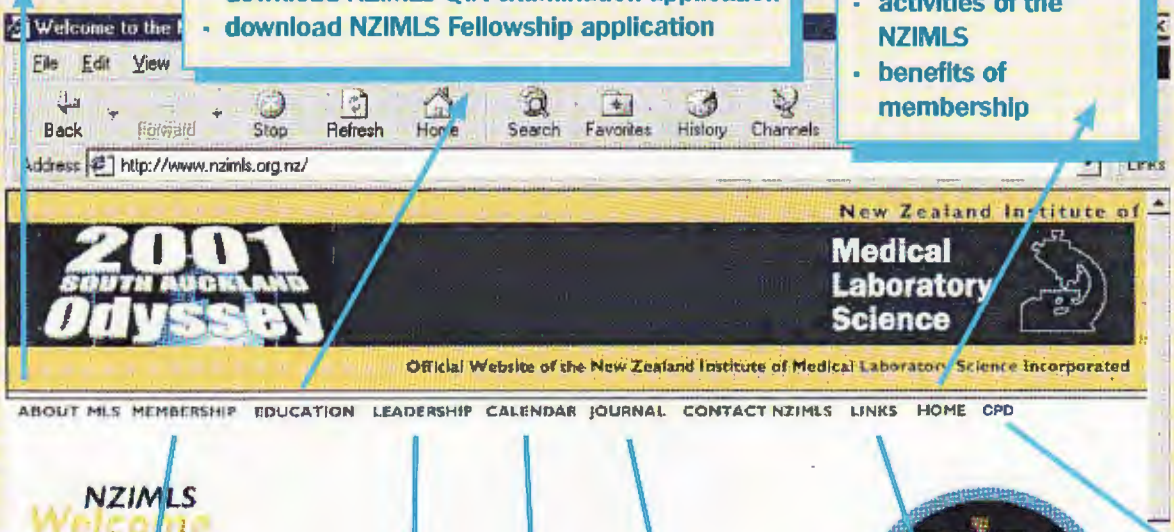

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