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Editorial

The Why, How, and What of Abstracts

Rob Siebers
Wellington School of Medicine

In this issue of the Journal, abstracts of presentations at the Annual Scientific Meeting of the NZIMLS are published, as they are each year. The prime reason for publishing these abstracts is education, as many of our colleagues are not able to attend Conference or the presentation, they will at least be able to learn what has been presented. Printing the Journal is not cheap, and the abstracts take up many pages (18 in this issue). Therefore it is paramount that what is printed, is of potential interest and use to members of the Institute.

A good abstract of a scientific study or case presentation should ask, and answer the following:

- **Why** was the study done (Background, or Introduction)
- **How** was it done (Methods)
- **What** were the main results (Results)
- **What** are the main conclusions (Conclusions)

An informative abstract is a mini-paper in itself, and writing an abstract with a fixed length is not easy and requires much thought and often many rewrites before it is acceptable. Unfortunately, many of the abstracts this year were deficient in one or more of the above. Indeed common statements in abstracts this year, and in preceding years are "Results will be presented" or "Results will be discussed". These statements may be OK for delegates at Conference as they will most likely attend the presentation if the title and subject speciality is of interest to them, and will then hear the results and conclusions. They are absolutely useless to readers of the Journal who were unable to attend the presentation. As Editor, I have to seriously ask whether it is of use to continue to waste valuable Journal space on such useless information.

Each year there are some abstracts that are properly structured. Looking through this year's abstracts, six New Zealand abstracts met my criteria for a good and informative abstract. Interestingly five of the six were from the microbiology/virology specialities.

- **Primary Amoebic Meningo-Encephalitis Caused by Naegleria fowleri.** *Matthew Akehurst, Microbiology Department, Health Waikato, Hamilton.*
- **Viral meningitis outbreak due to Echovirus type 33.** *D Hulston, J Shewan, S Huang, ESR, Porirua; J Lindeman, Waikato Hospital; and MC Croxson, Auckland Hospital.*
- **Human Cytomegalovirus and Epstein-Barr Virus: Double Trouble for Serologists.** *Paul Austin, Department of Virology & Immunology, LabPlus, Auckland Healthcare Services.*
- **Coughing Up a Storm.** *Mirjam Horsburgh, Medlab South, Christchurch.*
- **Extended-spectrum Beta-lactamases Among Urinary Escherichia coli and Klebsiella spp. In New Zealand in 2000.** *Maggie Brett and Rosemary Stanley, ESR, Porirua.*
- **The Effect of Haemolysis on Biochemistry Results.** *Don Mikkelsen and Stephen Tung, Biochemistry Department, Waikato Hospital, Hamilton.*

Three of the above New Zealand presenters are current financial members of the NZIMLS and are (surprise) winners of the Editor's prize

for excellence in abstract writing. Prizes have been sent to D. Hulston, ESR, Porirua; P Austin, LabPlus, Auckland; and R. Stanley, ESR, Porirua. Hopefully, this may spur other members on to produce informative abstracts for next year's ASM in Auckland. Use the above mentioned as good examples on how to write up an abstract, or see a previous article on the subject published in the Journal (1).

Reference

1. Siebers RW, Elliot J. Abstract writing for scientific meetings. *N Z J Med Lab Science* 1993; 47:6.

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Effects of storage at 4° C for seven days on ten serum analytes.

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Abstract

Sample stability depends on storage temperature and will vary with the specific analyte in serum. Therefore stability is an important factor to consider when extra tests are performed on samples that have been refrigerated. In an attempt to determine sample stability, based on the current analytical methods used within the laboratory, we examined the effects of storage at 4°C for 7 days on 10 commonly requested tests.

Blood samples collected from 15 male subjects were centrifuged and serum was left on the clot. The concentrations of Free-thyroxine, Free-triiodothyronine, Thyroid-stimulating hormone, Folate, Vitamin B12, Ferritin, Prostatic-specific antigen, Cortisol, Iron, and Unsaturated Iron Binding Capacity were measured at 0, 3, 5 and 7 days of storage at 4°C. The mean percentage change for each analyte was calculated and plotted. The criterion for determining the stability of the analyte was the run-to-run precision of the assay.

Serum specimens to be tested for concentrations of Free-thyroxine, Free-triiodothyronine, Vitamin B12, Folate, and Unsaturated Iron Binding Capacity could be stored for one week refrigerated without any loss of analyte activity. Stability was acceptable for five days on samples for Thyroid-stimulating hormone, Ferritin and Iron stored at 4°C. Specimens for Prostatic-specific antigen and Cortisol could not be stored at 4°C for longer than 24 hours.

Key words

Storage temperature, sample stability, clinical biochemistry, serum.
NZJ Med Lab Science 2000 ;54(3): 83-86

Introduction

A general problem in clinical laboratories is the stability of analytes during sample storage. Knowledge of sample stability on stored specimens is an important factor to be considered for all assays. Our laboratory receives as many as 40 requests per week for extra tests to be performed on stored blood specimens. Requesting a fresh sample every time an extra test is required is not always practicable, thus the blood specimens are centrifuged and stored under local conditions (4°C) for a period of one week. Several phenomena could occur during sample storage. Some analytes could decrease and others increase due to enzymatic cleavage of precursor molecules. Prolonged contact of serum with the clot can lead to an increase or decrease of analyte concentration in the serum (1). Therefore it is critical to know that the reported test results from stored specimens truly reflect in vivo analyte levels. Previous studies have shown that sample stability depends in part on storage temperature, and will vary with the specific analyte in serum (1-5). However information on the stability of the serum ana-

lytes during storage is often based on particular methods and sometimes is incomplete and contradictory.

In this study we reviewed the stability of some commonly requested analytes in specimens that have been stored under local conditions (4°C), based on current analytical methods used within the laboratory, in an attempt to make recommendations regarding 'extra testing' in specimens which have been stored for up to 7 days. Our criterion for deciding stability was based on technical variation, although biological variation also needs to be considered.

Materials and methods

Apparatus

Iron studies (Fe and UIBC) were determined with a Hitachi 917 analyser (Roche), while the other analytes were determined with an ACS: 180 analyser (Chiron).

Sample collection and handling

Venesection was performed on 15 apparently healthy male volunteers (age range between 30-50 yrs), with their informed consent, between 0930 hours and 1200 hours. All 15 samples were tested and found to be within the normal reference range. The venous samples were collected in 10-ml normal vacutainer sample tubes without any anticoagulants.

After allowing the samples to clot for 2 hours at room temperature, all the blood samples were centrifuged for 10 minutes at 4000g and serum left on the clot. Each serum was then assayed for the following analytes: Free-thyroxine (FT4), Free-triiodothyronine (FT3), Thyroid-stimulating hormone (TSH), Folate, Vitamin B12 (VB12), Ferritin, Prostatic-specific antigen (PSA), Cortisol, Iron (Fe), and Unsaturated Iron Binding Capacity (UIBC). Day 'zero' value served as the reference value.

Measurements of the analytes examined were repeated after 3, 5, and 7, days of storage of the sera at 4°C. To avoid evaporation samples tubes were kept closed until analysis. The Hitachi 917 and ACS: 180 were calibrated according to the routine procedures of the laboratory. Quality control samples at low and high levels of analytes were incorporated throughout the study to ensure that the analyser was running within laboratory specification.

Reagents and methods

FT4, FT3, TSH, Ferritin, Folate, VB12, PSA, Cortisol, were assayed by chemiluminometric immunoassay using reagents supplied by Chiron Diagnostics Ltd (USA). Fe and UIBC were measured by colourimetric assay with Ferrozoin without deproteinization at pH 5.0 with reagents purchased from Roche (New Zealand).

Calculations

The changes of the values after storage (for 3, 5, and 7 days) were cal-

culated for each subject as percentages of day zero values (the latter being assigned a value of 100%). The mean percentage change for each analyte was calculated and plotted. Criterion for analyte stability was the run-to-run precision of each assay (%2CV).

Results

Our criterion for analyte stability was the run-to-run precision of each assay. If the results obtained for the one-week period of the study remained within the 2CV limit of the assay, they were considered to be technically indistinguishable from the initial assay values. The changes in the measured analytes are shown as percentages of the initial value in Figures 1-10. The overall mean value on day zero, the mean percentage change of the analyte level on day 3, 5, and 7, the analytical variation of the assay, and the intra-individual variation (CVI), for the ten analytes that were studied are shown in Table 1.

According to Fraser (6) estimates of intra-biological variation are similar irrespective of time span of the study, number of subjects studied, place of study, analytical methodology and whether the subjects are healthy, young or old, or have stable chronic disease. Therefore we were able to use the collated data by Fraser (6) on biological variation for the purpose of our study.

Most of the serum analytes (FT4, FT3, VB12, Folate and UIBC) measured were unaffected by storage of serum with the cell clot at 4°C (Figures 1, 2, 3, 4 and 5 respectively), since the observed variations in the concentrations of the samples between the test results for the initial day and after storage for one week were within the expected day-to-day precision of the analytical methods used. The mean percentage changes on day seven for TSH, Ferritin, and Iron (Figures 6, 7 and 8 respectively) were outside the 2CV limit of the assay.

While TSH and Ferritin showed a 16% and 11% increase on day 7 respectively, there was a sharp increase in the concentration of Iron (almost a 25% increase from the initial values). A consistent increase in activity of Cortisol was observed after 24 hours of storage at 4°C (Figure 9). PSA activity declined after 7 days of storage (Figure 10). The mean percentage change for day 2 was on the acceptable limit but appeared to be within the day-to-day precision of assay on day 5.

Table 1. Components of variation. mean percentage change of analyte level on days 3, 5, and 7.

Analyte	Analytical variation (% 2CV)	Intra-individual Variation (%CVI) ^c	Mean day zero value (100% value of Fig. 1- 10)	Mean percentage (%) change on days		
				3	5	7
FT4	19.4	9.5	15.93 pmol/L	4.44	15.75	16.72
TSH	14.82	16.2	2.19 mIU/L	1.84	5.67	16.25
FT3	8.1	7.9	4.99 pmol/L	1.62	2.05	1.65
Ferritin	8.1	14.72	173.8 µg/L	0.53	3.11	10.81
VB12	14.44	†	298.53 pmol/L	2.09	5.53	7.61
Folate	18.6	†	17.63 pmol/L	2.44	10.53	14
PSA	18.2	18.1	1.58 µg/L	18.76	10.84	20.7
Cortisol	8.46	15.2	378.66 nmol/L	14.22	16.21	28.82
Fe	6.16	15.9	19.27 µmol/L	2.33	2.13	23.93
UIBC	10.16	†	38.93 µmol/L	1.01	2.91	0.01

*CVI based on data collated by Fraser (6)

†CVI data not found in literature

Discussion and conclusion

In stability studies one routinely seeks differences in measurements made before and after sample storage. In this study we decided to look at the relative percentage change of serum analytes over a set period of time. In practical terms the size of detectable differences is related to the precision of the method of measurement. According to Ralph et

al. (7), a constituent is stable under specified conditions when measurements demonstrate, with pre-specified risk of decision error, that its mean concentration in the tested specimens has changed by less than an amount, β where β is a function of the precision of the method (7). Based upon this definition, we decided to take the day-to-day precision of the assay as our criterion to evaluate analytical stability in stored specimens. If results obtained in the time span of the study (for one week) remained within the 2CV limit of the analytical method used, they were considered to be technically indistinguishable from the initial assay values. Since the CV is an indication of the standard deviation as a percentage of the mean it easily fitted in to the frame of our data comparison, where we assumed the mean to be 100%.

We were able to easily plot lines for plus or minus 2CV and get a visual representation of the region in-between where the values would fall 95.5% of the time if no change has occurred during storage. By setting our criterion of stability on the 2CV limit of the analytical method used, there was a 95.5% chance that an analyte would be stable if its measurement value was in-side the technical precision, and a 4.5% risk of error for our decision (i.e., there is a 0.045 probability of deciding that an analyte is stable when it is in fact unstable). The decision to take the 2CV range of the assay as our criterion for analyte stability was an arbitrary choice. Because most of the analytes tested in this study have large biological variations (due to either loose homeostatic control mechanisms or lack of attention to preanalytical variation) there was little point in being too conservative and reducing our decision point to 1CV.

Data on biological variation are quantitative estimates of the homeostatic control mechanisms of a single animal species (9). Therefore data on the intra-individual biological variation of analyte concentrations helps to assess the true significance of changes in results obtained for serial specimens from a single patient.

The current recommendation of an acceptable time period for the storage of most of the analytes we studied at 2-8°C is 24 hours (8). Kubasik et al. (3) previously described that Folate values are quite sensitive to storage conditions and VB12 as quite a robust analyte which is stable at different storage conditions for a minimum of two weeks. Based on the analytical methods used within our laboratory FT4, FT3, VB12, Folate, and UIBC concentrations were within the day-to-day precision of the assay after 7 days of storage at 4°C, thus indicating that the storage of samples for longer than 7 days at 4°C added little variation to the assay results.

According to our data Ferritin levels were technically unacceptable after day 5 of storage. Although the mean percentage change of TSH on day 7 (16.26%) was very close to the 2CV limit (14.82%) of the assay, there was only a 4.5% probability that TSH concentration was not increased above the acceptable limit value and a 95.5% probability that it was actually unstable. Therefore we decided that TSH values would be technically unacceptable after day 5. However it is interesting to note that these findings may bear little relevance to practical clinical decision making, since the variation of Ferritin and TSH on day 7 were inside the intra-individual biological variation of both analytes (Ferritin 14.72% CVI and TSH 16.2% CVI).

The sharp increase in Fe concentration observed on day 7 (23.93% from the initial value) was technically significant because it had increased above the set acceptable technical variation (6.16%). The mean percentage change of Fe on day 7 had also increased above its intra-biological variation (14.72%). Therefore if an individual's Fe levels were to be assessed on a sample that was stored beyond 5 days and were compared with the patient's previous results, the increase in the Fe level due to storage effects would be interpreted as true increase in the in-vivo Fe level. The package inserts that accompany the commercial reagent kit for Fe detection, indicates that samples could be stored

for 7 days at 4°C. However in light of our observations we recommend that Fe measurements should not be performed beyond 5 days of storage at 4°C.

The increase in the concentration of Cortisol after 24 hours of storage at 4°C (almost 29% increase on day 7 from the initial value), was technically significant. Also it is noted that the mean percentage changes of Cortisol after three days of storage fell outside the intra-biological variation of Cortisol (15.2 % CVI), thus Cortisol measurements performed on samples after 3 days of storage would result in assay values that are not representative of an individuals' true analyte level.

After 24 hours of storage the percentage change in PSA levels was greater than the intra-biological variance (18.1%) and the 2CV limit (18.2%) of the assay, indicating that the assay values for PSA may not reflect the in-vivo analyte level due to the effects of storage.

During sample storage some analytes showed an increase, others a decrease in concentration. These observed changes may be due to a pH change in specimens, which would interfere with the dynamics of the analytical methods used. There is a possibility that the structure of an analyte could change due to denaturing, thus exposing or masking antigen binding sites for antibody in the immunoassay methods. Microfibrin formation in the samples during storage could also interfere with the analytical method used to assay the analytes. Due to these storage effects on serum analytes, assay values may not reflect the true in-vivo analyte level. However changes in the concentration of analytes during storage may bear little relevance to practical clinical decision making depending on whether the analyte concentrations fall within the set reference interval. Information defining the acceptable maximum storage periods with respect to clinical decision making is either nonexistent or vague. Therefore we assessed sample stability based upon the precision of the analytical methods used within our laboratory.

This study did not include abnormal samples therefore we can not assume that abnormally high or low samples would behave similarly to normal samples. However when looking at the raw data of the analyte concentrations, there were no internal trends (i.e., the daily changes were independent of actual analyte concentrations). Therefore we can draw a tentative conclusion that the abnormal samples would behave similarly to the normal samples. To further verify this conclusion we would need to carry out a similar study that includes abnormal samples along side the normal samples.

Based on the observations made in our study we recommend, that specimens for PSA and Cortisol should not be stored for longer than 24 hours at 4°C and specimens for TSH, Ferritin, and Fe no longer than 5 days at 4°C. Specimens to be tested for concentrations of FT4, FT3, VB12, Folate, and UIBC can be stored at 4°C for a minimum of 7 days.

Acknowledgements

I thank Roger Barton, Trevor Rollinson and the staff at Southern Community Laboratories, Dunedin for their assistance in this research paper.

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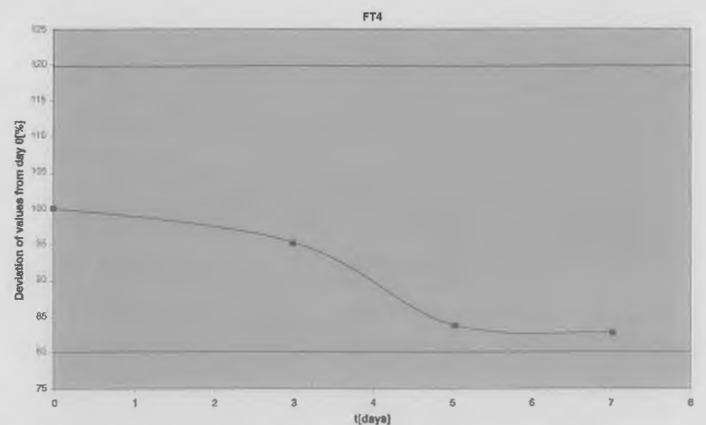


Figure 1. Percentage deviation of FT4 values from day 0

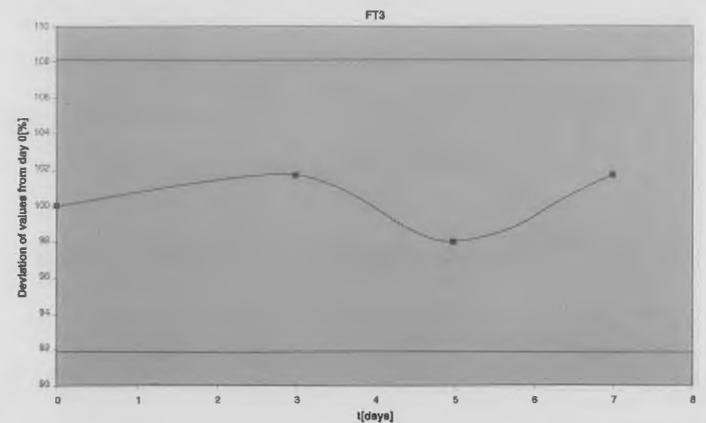


Figure 2. Percentage deviation of FT3 values from day 0

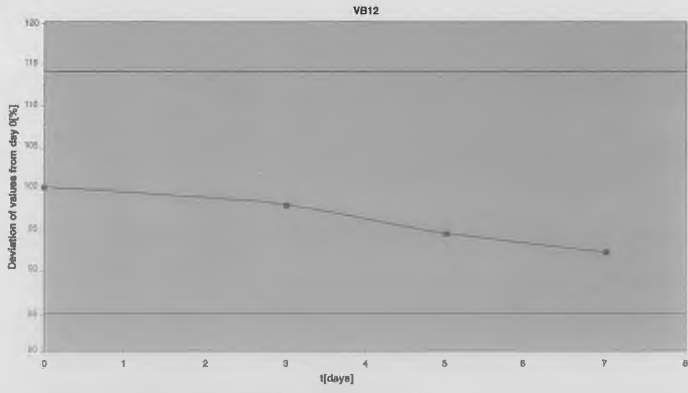


Figure 3. Percentage deviation of VB12 values from day 0

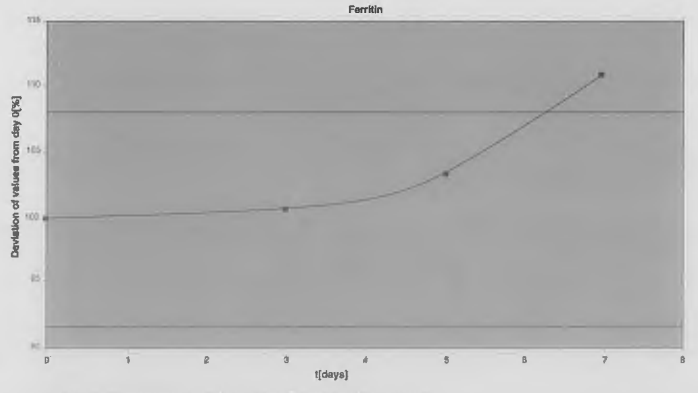


Figure 7. Percentage deviation of Ferritin values from day 0

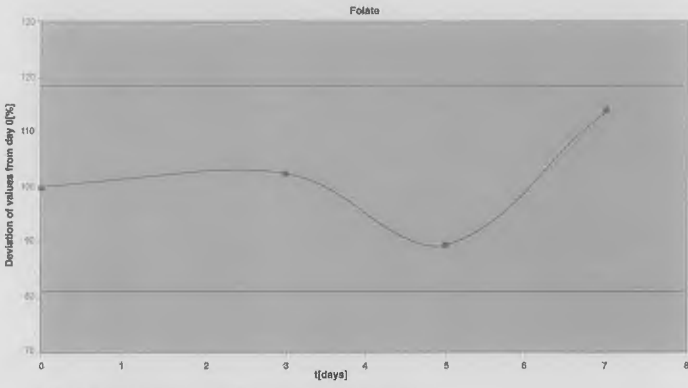


Figure 4. Percentage deviation of Folate values from day 0

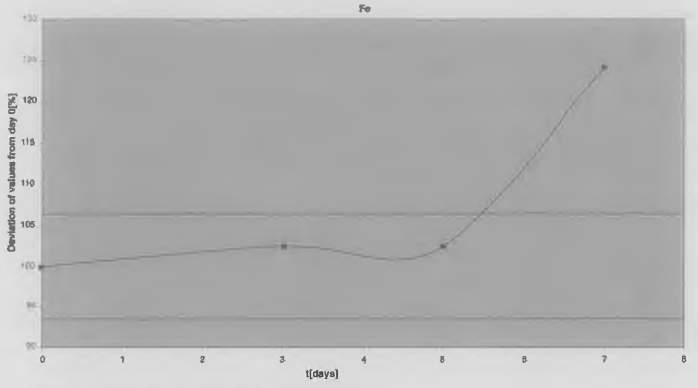


Figure 8. Percentage deviation of Fe values from day 0

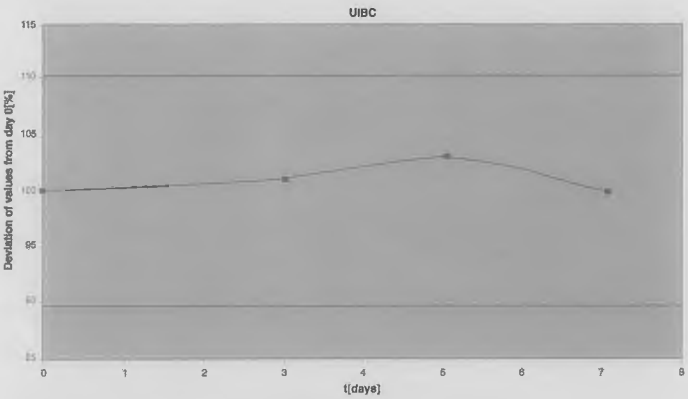


Figure 5. Percentage deviation of UIBC values from day 0

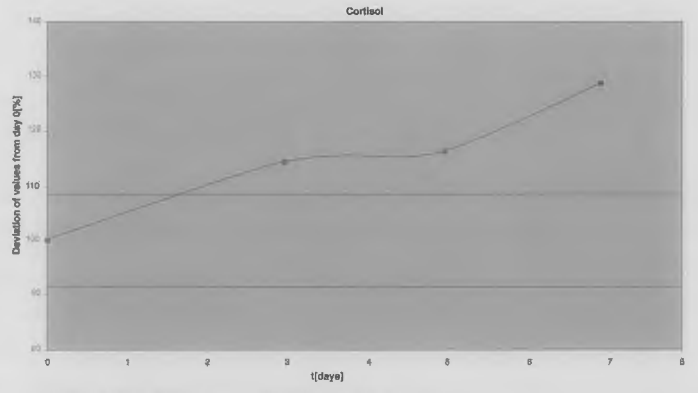


Figure 9. Percentage deviation of Cortisol values from day 0

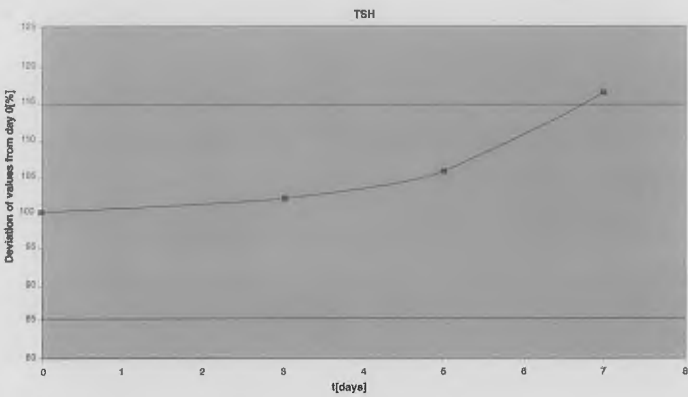


Figure 6. Percentage deviation of TSH values from day 0

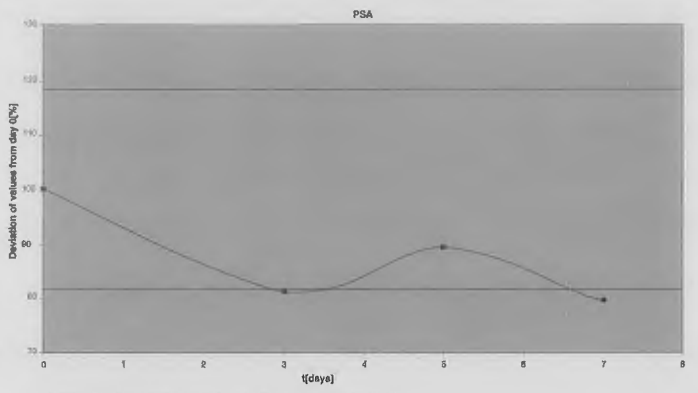


Figure 10. Percentage deviation of PSA values from day 0

A review of anaerobic microbiology at Health Waikato laboratory

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Abstract

In March 1995 a retrospective audit was undertaken to investigate the number and types of anaerobes that were being isolated at the Health Waikato Microbiology laboratory. Analysis showed a low anaerobe isolation rate (2.5%) and a low frequency of isolation of the more fastidious species of anaerobes. Major areas targeted for review included types of specimens, transport systems, primary isolation media, incubation systems, identification procedures and sensitivity methodology.

This study resulted in the implementation of changes over an eight-month period and a significant improvement in both the anaerobe isolation rate (from 2.5% to 37%) and in the types of anaerobes being recovered. In addition, the introduction of sensitivity testing also allowed the monitoring of resistance on clinically significant anaerobe isolates, thus providing useful information for empiric therapy choices within Health Waikato hospital.

N Z J Med Lab Science 2000; 54(3): 87-93

Introduction

Anaerobic bacteria are important pathogens, causing a wide variety of infections throughout the body. Infections caused by anaerobes are often polymicrobial and can be easily overlooked due to growth of other aerobic and facultative pathogens. This coupled with the belief that most anaerobic infections can be adequately treated with a combination of available antibiotics and / or surgical intervention has caused anaerobic microbiology to become a neglected area in some laboratories.

However, resistance to some of the most effective anaerobic antimicrobials is starting to appear in some parts of the world and providing clinicians with susceptibility data on patient isolates can influence choices regarding selection of appropriate therapeutic agents. A full and definitive identification of anaerobes can also provide useful clinical information e.g. there is a strong association between *Clostridium septicum* and malignancy and other disease of the colon, especially the caecum.

Health Waikato Microbiology laboratory was processing an average of 4400 specimens per month and as anaerobes were regularly recovered from clinical specimens it was assumed that current techniques for anaerobic culture were adequate. A retrospective audit showed that the anaerobic isolation rate was extremely low and that only the hardier anaerobes were being recovered.

The decision was made to review the area of anaerobic microbiology with the goal to be able to generate more accurate and reliable results that would be useful to the clinician for treatment of the patient.

This study discusses the areas targeted for review, implementation of new methodologies and the impact that these changes have had on the anaerobic isolation rate at Health Waikato Microbiology laboratory.

Materials and Methods

Collection and transport of specimens

Information sheets were prepared and sent to medical and nursing staff outlining the new requirements and recommendations for the collection and transport of specimens requiring anaerobic culture. Emphasis was placed on the importance of collecting specimens in order to minimise contamination with indigenous anaerobic flora and to avoid the generation of misleading culture results (1).

The following specimens were listed as acceptable for anaerobic culture (1,2).

- Fluid or aspirate e.g. from an abscess obtained during a surgical procedure, after surface decontamination.
- Biopsy material surgically obtained.
- Anaerobic swab surgically obtained when aspiration is not feasible.
- Transtracheal aspirate.
- Material from percutaneous lung puncture.
- Deep aspirate of open soft tissue wound or ulcer obtained through decontaminated adjacent skin.
- Blood culture.

A crucial factor affecting the success of anaerobic cultures is the transport of specimens (1,3). There were no protocols in place, or transport media available, for submitting specimens to the laboratory for the culture of anaerobes. After reviewing information on a selection of products, the decision was made to purchase an anaerobic transport medium (ATM) manufactured by Anaerobe Systems.

This ATM system consists of a 10 cm transparent tube, with a screw cap containing a rubber septum. Inside the tube is a long column of media consisting of buffered mineral salts in a soft agar base with sodium thioglycolate and cysteine added to provide a reduced anaerobic environment. Small pieces of tissue, and swabs, can be pushed down into the medium and up to 7 ml of fluid is able to be directly injected into the tube.

The ATM is stored at room temperature with a shelf life of up to one year. Quality control information sheets are received with each batch. This media is held in the laboratory and issued to the wards on request. A small stock ATM is also maintained in theatre. Instruction sheets outlining the procedure for inoculation of the media are included with every pack issued. This ATM is also suitable for the recovery of aerobic and microaerophilic organisms.

Inoculated ATM should be kept at room temperature during transport to the laboratory and up until the time of culture. It has been shown that the number of viable anaerobic organisms decreases at refrigeration temperatures due to the increased absorption or diffusion of oxygen (3).

Large volumes of purulent fluid (2 ml or greater) and large pieces of tissue may not require special anaerobic transport conditions, as they appear to contain reducing substances that will maintain the viability of any anaerobes present in the specimen for a few hours (2,3).

The acceptable length of time between specimen collection for anaerobic culture and transport to the laboratory is no longer than two

hours. However, this time can vary depending on the quantity and nature of the specimen and whether an anaerobic transport system has been used or not (2,3).

Blood culture specimens collected on adult patients (i.e. > 14 years) include both an aerobic and anaerobic culture bottle. The anaerobic culture bottle is adequate for supporting the growth and maintaining the viability of anaerobes for the duration of the six day processing protocol. Blood culture specimens were processed using the VITAL analyser (bioMerieux Vitek).

Primary isolation media

Evaluation of the primary isolation media used for anaerobic cultures was performed by comparing the growth of anaerobic bacteria on pre-reduced anaerobically sterilised (PRAS) plated media with growth on current in-use media.

The PRAS media used was manufactured by Anaerobe Systems. A three-plate package of PRAS media was chosen containing:

- Brucella blood agar + vitamin K + haemin (BRU).
- Phenylethyl alcohol blood agar (PEA).
- Bacteroides bile esculin (BBE) / Laked blood kanamycin vancomycin (LKV) split plate.

This PRAS media is prepared in an anaerobic environment and is packaged in pre-reduced oxygen free foil envelopes. Each unopened package has a shelf life of up to three months stored at room temperature. All PRAS media is received in the laboratory with quality control information sheets.

The in-use media was produced and supplied by Fort Richard Laboratories Ltd. (P.O. Box 22172, Auckland, New Zealand). This media was not pre-reduced or anaerobically sterilised. The supplier performed quality control on the media prior to its release to the laboratory. The bulk of this media was stored at 4°C, with stacks of plates also kept on the bench, at room temperature, for ease of use.

Three types of media were currently in-use

- Wilkins chalgren agar (WC).
- Vancomycin / kanamycin agar (VK).
- Neomycin agar (NEO).

Six quality control organisms were chosen for use in this evaluation. These organisms included:

- | | |
|--|------------|
| • <i>Bacteroides fragilis</i> | ATCC 25285 |
| • <i>Bacteroides levii</i> | ATCC 29147 |
| • <i>Clostridium perfringens</i> | ATCC 13124 |
| • <i>Fusobacterium necrophorum</i> | ATCC 25286 |
| • <i>Fusobacterium nucleatum</i> | ATCC 25586 |
| • <i>Peptostreptococcus anaerobius</i> | ATCC 27337 |

These control strains were obtained from Anaerobe Systems. Isolates were received in ATM and subcultured onto PRAS BRU media and incubated at 35 °C for up to 7 days, or until visible growth was observed. The two *Fusobacterium* isolates appeared to be non-viable on subculture from the ATM. To determine whether this was due to the incubation system, the media used, or whether the organisms were non-viable on receipt, the original ATM containing these two isolates was submitted to Auckland Laboratory for culture. It was concluded that these two isolates were non-viable after Auckland laboratory was also unsuccessful in subculturing the organisms. The decision was made to continue the evaluation without these two isolates and review the results obtained with the other quality control strains.

The remaining four quality control organisms were suspended in 0.85 % NaCl to the turbidity of a 0.5 McFarland standard. These suspensions were then diluted 1:100 with 0.85 % NaCl. 10 µl aliquots of each 1:100 diluted suspension (approximately 10³-10⁴ CFU / ml) were then inoculated onto a set of PRAS media and in-use anaerobic media.

Table 1. Quality control organisms used in anaerobic biochemical evaluation

Gram negative anaerobes	Gram positive anaerobes
<i>Bacteroides fragilis</i> group	<i>Clostridium</i> species
<i>Bacteroides fragilis</i>	<i>Clostridium perfringens</i>
<i>Bacteroides thetaiotamicron</i>	<i>Clostridium difficile</i>
<i>Bacteroides vulgatus</i>	<i>Clostridium bifermentans</i>
<i>Bacteroides distasonis</i>	<i>Clostridium sordellii</i>
<i>Bacteroides ovatus</i>	<i>Clostridium sporogenes</i>
Other <i>Bacteroides</i> species	<i>Propionibacterium</i> species
<i>Bacteroides ureolyticus</i>	<i>Propionibacterium acnes</i>
	<i>Propionibacterium granulosum</i>
<i>Prevotella</i> species	<i>Peptostreptococcus</i> species
<i>Prevotella melaninogenica</i>	<i>Peptostreptococcus asaccharolyticus</i>
<i>Prevotella intermedia</i>	<i>Peptostreptococcus anaerobius</i>
<i>Fusobacterium</i> species	<i>Eubacterium</i> species
<i>Fusobacterium nucleatum</i>	<i>Eubacterium lentum</i>
<i>Fusobacterium necrophorum</i>	
	<i>Actinomyces</i> species
	<i>Actinomyces israelii</i>

In accordance with manufacturers recommendations the PRAS plate media was not exposed to air for more than five minutes before inoculating and the inoculated plates were not exposed to air for more than 10 minutes before incubating. Plates were incubated in anaerobic jars and examined daily, for a total of five days.

A standard anaerobic workup on a clinical specimen included the use of a cooked meat / glucose broth, supplied by Fort Richard Laboratories. Due to the size of batches produced and long expiry dates, these cooked meat / glucose broths could be several days, or even weeks old, when received in the laboratory.

For best results, this broth should be inoculated as soon as possible after preparation (4). Therefore prior to use, all broths are free-steamed at 100°C, with loosened caps, in an autoclave, for 10 - 15 minutes to remove dissolved oxygen (5). Frequent processing of small batches of these broths by laboratory staff ensure they are as fresh as possible prior to inoculation with clinical material.

Incubation system

The existing anaerobic incubation method consisted of an anaerobic jar system with all anaerobic culture plates being incubated for a total of 48 hours. An anaerobic atmosphere was generated inside the jars using catalysts consisting of palladium-coated alumina pellets (Don Whitley Scientific Limited) in conjunction with the Gas-Tek™ Anaerobic System (Remel). Conditions in the jar were monitored by the addition of Oxoid Anaerobic Indicators. These indicators contained resazurin and changed from pink to colourless in low concentrations of oxygen.

After use catalysts were dried thoroughly in a hot air oven at 160°C for 30 - 60 minutes and stored in a dry area (1,6). This was to avoid partial or total inactivation of the catalyst due to moisture produced during incubation of the anaerobic jars.

As laboratory staff regularly noted "failures" of this system, the routine maintenance procedures for the anaerobic jars and catalysts were investigated and found to be inadequate. The O ring seals on many of the jars were not in good condition and a lubricant was frequently being used to help seal the jars. The manufacturer (Don Whitley Scientific Ltd.) did not recommend this practice.

The use of excessive pressure on the lid clamps had distorted and cracked many of the plastic lids and these cracked lids were still being regularly used. Manufacturer recommendations suggested routine replacement of the catalysts should be undertaken after using for approximately 90 cycles i.e. 3 months, however these catalysts were being continuously recycled.

It is now recognised that exposure of the palladium catalyst to certain products of anaerobe metabolism e.g. hydrogen sulphide and volatile fatty acids can result in irreversible poisoning of the catalyst (7,8). Heating will not restore the activity of the catalyst. Anotox™ (Don Whitley Scientific Ltd.) is a specially impregnated activated carbon designed to absorb hydrogen sulphide and a range of volatile fatty acids produced by anaerobic bacteria (7,8). Therefore, inclusion of Anotox™ in any anaerobic incubation system using the palladium catalyst would substantially prolong the life of the catalyst (7). Prior to this study Health Waikato laboratory was not using Anotox with the palladium catalysts.

A number of alternative anaerobic incubation systems were looked at e.g. Anaerobic Work Stations (Don Whitley Scientific Ltd.) and GasPak Pouch™ System (BBL), but the decision was made to continue with an anaerobic jar incubation system. Factors influencing this decision were cost, number of cultures performed and space limitations within the laboratory.

A literature review suggested that improvements should be made to the way the anaerobic jar incubation system was being used (1,6-9). This resulted in the purchase of new anaerobic jars. The jars chosen were the 2.5 liter AnaeroJar™ (Oxoid), to be used in conjunction with the AnaeroGen™ (Oxoid) anaerobic atmosphere-generating system. This system requires neither catalyst nor water and therefore reduces preparation time to a minimum. The AnaeroGen™ sachet is activated on contact with air. A disposable indicator strip must be added to the system and the Oxoid Anaerobic Indicators are used.

It was decided all culture plates should be held for a total of 7 days before discarding as negative (5). Cultures are initially reviewed after 48 hours incubation at which time an interim report is issued.

Anaerobic biochemical identification systems

The performance of the in-use VITEK ANI card (bioMérieux Vitek) was evaluated by testing it against a selection of quality control strains and in parallel with the RAPID ID 32, an identification system for anaerobes (bioMérieux).

For both the VITEK ANI card and the RAPID ID 32A anaerobe identification systems, testing is based on enzymatic degradation of specific substrates, which is detected by a variety of indicator systems.

A total of 22 organisms were selected for testing from the major groups of anaerobes commonly encountered in clinical specimens (Table 1). Lyophilised cultures of these organisms were obtained from the Institute of Environmental Science and Research, Communicable Disease Center (P.O. Box 50-348, Porirua, New Zealand). Cultures were reconstituted according to instructions received with each organism, inoculated onto non-selective PRAS BRU media and incubated in an anaerobic jar system, until visual growth was detected. Cultures were further subcultured onto PRAS BRU agar and incubated anaerobically for a further 48 - 72 hours, prior to inoculation of the two identification systems.

A minimum of a 3.0 McFarland turbidity suspension of each organism was prepared in 1.8 ml of 0.45 % NaCl for inoculation of the VITEK ANI card. The card was filled and sealed in the VITEK filler / sealer unit and incubated vertically, in aerobic conditions, at 35°C for four hours. Supplemental off-line testing was required to be performed on all isolates being identified using the ANI card. These tests included Gram stain reaction, morphology and spot indole test. The reagent used for

the spot indole test was p-Dimethylaminocinnamaldehyde. After completion of incubation the colour reactions for each well on the ANI card were read manually and recorded in the VITEK analyser, along with the supplemental test results. Results were then analysed automatically using the VITEK (software version R03.01).

A 4.0 McFarland turbidity suspension of each organism was prepared in 2 ml of sterile distilled water for inoculation of the RAPID ID32 A system. A 55 µl aliquot of suspension was dispensed manually into each cupule and mineral oil was added to the appropriate wells. The strips were incubated in aerobic conditions, at 35°C for 4 hours. Reagents were then added to select tests before the strips were read manually and the numerical profile numbers were calculated. The ten digit profile number was then analysed using the API Lab Plus (software version 3.0).

Sensitivity testing

The Etest (AB Biodisk) methodology was introduced for sensitivity testing of anaerobes. The Etest is a rapid and reliable method for determining the minimum inhibitory concentrations (M.I.C.'s) of anaerobes to a wide range of antibiotics and shows good categorical agreement with the agar dilution reference method (10-12).

NCCLS recommend the use of supplemented BRU for agar dilution procedures as many anaerobes grow poorly in both microdilution and agar dilution tests using Wilkins-Chalgren broth or agar (10,12,13). PRAS BRU medium, containing haemin and vitamin K, was chosen for use. This media is produced by Anaerobes Systems and is received in pre-reduced oxygen free foil envelopes. Each unopened package has a shelf life of up to three months stored at room temperature.

Antibiotics tested routinely include metronidazole, clindamycin, cefoxitin and amoxicillin/clavulanate. Imipenem is tested on request. The culture used for testing can be 24, 48 or 72 hours old, depending on the growth rate of the organism. A 1.0 McFarland turbidity suspension is required for inoculation of the PRAS BRU plates.

The duration of incubation is dependent on the growth rate of the test isolate. If sufficient growth is seen after 24 hours incubation the plates can be read. The antibiotic tested can also influence the length of incubation. For example, a clindamycin sensitivity result can only be finalised after 48 hours incubation.

False resistance to metronidazole has been reported when testing has not been performed under anaerobic conditions (14). To ensure that sub-optimal anaerobiosis is not occurring during incubation, a quality control strain, as recommended by NCCLS must be tested with each isolate (13). The quality control strain *Bacteroides fragilis* ATCC 25285 is put up against metronidazole and incubated in parallel with all test isolates. The minimum inhibitory concentration (M.I.C.) results of the test and control isolates are read and compared to NCCLS breakpoints and reference ranges (13). A result of either sensitive or resistant to each antibiotic tested is recorded on the laboratory report.

Results

Collection and transport of specimens

Rationalisation of specimens suitable for anaerobic culture resulted in a 91% decrease in the number of anaerobic cultures set up in the laboratory. One-thousand and eighty-seven specimens were cultured for anaerobes in the 14 month period prior to the implementation of these changes while only 248 specimens were processed in the subsequent 36 months.

Problems still occur with transport of specimens, particularly with extended delays between collection and receipt of specimens in the laboratory. The more commonly received specimens in the laboratory i.e. small pieces of tissue or biopsy material, swabs or small volumes of fluid are often not transported in ATM. Continued distribution of infor-

mation and on-going education of medical and nursing staff regarding the protocol for anaerobic culture of specimens is still required for improvement in this area.

Primary isolation media

Bacterial growth was quantified and recorded until sufficient growth for pure subculture was obtained i.e. individual colonies were visible on the agar surface. Growth was recorded past this time only if pigment production was expected and had not yet been observed. No growth on the selective agars tested was checked and recorded for the full five day period to verify that the selective agents remained stable in the media during extended incubation.

The *Bacteroides fragilis* and *Clostridium perfringens* quality control strains tested in this evaluation did not demonstrate significantly better growth on the PRAS media compared to the in-use media. This observation was not unexpected as these two strains are considered to be non-fastidious and more aerotolerant compared with many clinically encountered anaerobes. The two more fastidious strains tested i.e. *Bacteroides levii*, and *Peptostreptococcus anaerobius* showed more luxuriant growth and took less time to isolate visibly distinct colonies on the PRAS media compared to the in-use media.

Haematin and vitamin K supplements are included in the PRAS BRU and PEA media to enhance the recovery of black pigmented *Bacteroides* species. The *Bacteroides levii* strain produced black pigment on the PRAS BRU and PEA media, but no pigment was evident on the in-use media.

The PRAS selective / differential media worked well. PRAS LKV media is designed for the recovery of pigmented *Prevotella* species. Pigmented *Porphyromonas* and *Bacteroides* species are susceptible to vancomycin. (15). The growth of the *Bacteroides levii* control strain was successfully inhibited for the duration of incubation. The results obtained from the PRAS BBE demonstrated the usefulness of the medium for the rapid presumptive identification of *Bacteroides fragilis* group organisms. See Tables 2, 3, 4 and 5 for summary of results.

This demonstration of significantly better growth on the PRAS media with the two fastidious strains of *Bacteroides levii*, and *Peptostreptococcus anaerobius* led to the decision not to attempt to obtain repeat cultures of the two nonviable quality control strains of *Fusobacterium* species. It was assumed that the PRAS media would support the growth of the *Fusobacterium* species better than the in-use media would as had been demonstrated with the *Bacteroides levii* and *Peptostreptococcus anaerobius* strains. Validation of this decision can

Table 2. Comparison of growth of *Bacteroides fragilis* ATCC 25285 on PRAS media and in-use anaerobic media

	Day 1	Day 2	Day 3	Day 4	Day 5
PRAS Media					
BRU	‡	‡ §			
PEA	†	‡ §			
BBE	†	† §			
LKV	‡	‡ §			
In-use Media					
WC	‡	‡ §			
VK	‡	‡ §			
NEO	*	* §			

* Scanty growth. † Light growth. ‡ Moderate growth. § Pure subculture possible i.e. individual colonies visible. || Blackening of agar.

be seen in the results recorded in Table 6. Prior to implementation of the methodology changes, no *Fusobacterium* species were isolated. In the 36 months post implementation seven isolates of *Fusobacterium* were recovered including strains of *F. necrophorum*, *F. nucleatum* and *F. mortiferum*.

Table 3. Comparison of growth of *Clostridium perfringens* ATCC 13124 on PRAS media and in-use anaerobic media

	Day 1	Day 2	Day 3	Day 4	Day 5
PRAS Media					
BRU	† §				
PEA	‡ §				
BBE	NG	NG	NG	NG	NG
LKV	NG	NG	NG	NG	NG
In-use Media					
WC	† §				
VK	NG	NG	NG	NG	NG
NEO	† §				

† Light growth. ‡ Moderate growth. § Pure subculture possible i.e. individual colonies visible. NG = no growth.

Table 4. Comparison of growth of *Bacteroides levii* ATCC 29147 on PRAS media and in-use media

	Day 1	Day 2	Day 3	Day 4	Day 5
PRAS Media					
BRU	NG	‡	‡ §		
PEA	NG	‡	‡ §		
BBE	NG	NG	NG	NG	NG
LKV	NG	NG	NG	NG	NG
In-use Media					
WC	NG	NG	NG	NG	NG
VK	NG	†	†	† §	
NEO	NG	†	†	† §	

† Light growth. ‡ Moderate growth. § Pure subculture possible i.e. individual colonies visible. || Production of black pigment. NG = no growth.

Table 5. Comparison of growth of *Peptostreptococcus anaerobius* ATCC 27337 on PRAS media and in-use media

	Day 1	Day 2	Day 3	Day 4	Day 5
PRAS Media					
BRU	‡	‡ §			
PEA	†	† §			
BBE	NG	NG	NG	NG	NG
LKV	NG	NG	NG	NG	NG
In-use Media					
WC	†	† §	NG	NG	NG
VK	NG	NG			
NEO	*	†	† §		

* Scanty growth. † Light growth. ‡ Moderate growth. § Pure subculture possible i.e. individual colonies visible. NG = no growth.

Incubation system

No jar failures have been noted with the use of the AnaeroJar™ (Oxoid) and the AnaeroGen™ (Oxoid) anaerobic atmosphere generating system during this study.

Anaerobic biochemical identification systems

An acceptable identification of > 85 % probability was obtained with 14 (63 %) of strains tested using the VITEK ANI system. An identification probability of < 85 % was obtained with 3 (14 %) of the strains, but correct identification was confirmed by extra off-line tests such as catalase, pigment production, lipase or lectinase production. Of the remaining five isolates tested, 2 (9 %) gave test results that did not match any species group in the data base and 3 (14 %) gave an incorrect identification.

The RAPID ID 32 A system gave an acceptable identification of > 85 % probability with 15 (68 %) of the strains tested. Off-line testing was required to confirm the identification of 1 (5 %) isolate and 2 (9 %) isolates gave doubtful profiles. An incorrect identification was obtained for 4 (18 %) of the isolates tested.

A breakdown of the results obtained by correct identification percentage showed both systems gave identical results for the gram negative anaerobes. *Bacteroides vulgatus*, a member of the *Bacteroides fragilis* group of organisms, could not be identified by either system. *Prevotella melaninogenica* could not be identified by the VITEK ANI card and the RAPID ID 32A system could not identify *Prevotella intermedia*.

With the gram positive anaerobes the RAPID ID 32A system did not perform as well as the VITEK ANI card when tested against the *Peptostreptococcus* and *Propionibacterium* species. With exception of *Clostridium perfringens*, both systems were unable to speciate the *Clostridium* strains tested.

When the overall performance of both systems is collated, 17 (77 %) of the 22 quality control isolates were able to be correctly identified using the VITEK ANI card while the RAPID ID 32A system correctly identified 16 (73 %) of the isolates tested.

Based on the results of this evaluation the decision was made to continue to use the VITEK ANI.

Sensitivity testing

Since the introduction of Etest susceptibility testing in July 1995, 76 anaerobes have been tested. 26 (34%) of these isolates were recovered from blood culture specimens. A breakdown of isolates tested shows that 31 (41%) were from the *Bacteroides fragilis* group, 14 (18%) were other gram negative anaerobes including *Prevotella*, *Fusobacterium* and *Porphyromonas* species, 13 (17%) were *Clostridium* species and 18 (24%) were gram positive non-spore forming anaerobes including *Peptostreptococcus*, *Actinomyces* and *Propionibacterium* species.

Resistance has been detected to cefoxitin with 6 (13%) isolates from the *Bacteroides fragilis* group of organisms, and to metronidazole with 5 (28%) gram positive non-spore forming anaerobes. Resistance to cefoxitin and metronidazole with these types of anaerobes has been documented in the literature (10,16).

Numbers and types of isolates and analysis of specimen types

The number and types of anaerobes that have been isolated pre and post implementation of methodology changes are shown in tables 6 and 7. The anaerobe isolation rate has increased from 2.5% (27 isolates from 1024 specimens collected from 01 May 1994 to 30 June 1995) to 37% (91 isolates from 248 specimens collected from 01 July 1995 to 01 July 1998).

Table 6. Number and types of gram negative anaerobes isolated pre and post implementation of methodology changes

Organism	01 May 1994 - 30 June 1995 (14 months) number of isolates *	01 July 1995 - 01 July 1998 (36 months) number of isolates *
<i>Bacteroides</i> species †	9	3
<i>Bacteroides fragilis</i>	3	19
<i>Bacteroides fragilis</i> group organisms	2 ‡	10 §
<i>Bacteroides ureolyticus</i> group	0	1
<i>Fusobacterium</i> species	0	7 ¶
Pigmented <i>Prevotella</i> species	2	2
Other <i>Prevotella</i> species	0	5 **
<i>Porphyromonas</i> species	0	1

Excludes duplicate patient isolates. † Not speciated further. ‡ Two isolates of *B. stercoris*. § Isolates include *B. ovatus*, *B. thetaiotaomicron*, *B. uniformis* and *B. vulgatus*. ¶ Isolates include *F. necrophorum*, *F. nucleatum* and *F. mortiferum*.

** Isolates include *P. bivia*, *P. buccae* and *P. oralis*.

Table 7. Number and types of gram positive anaerobes isolated pre and post implementation of methodology changes

Organism	01 May 1994 - 30 June 1995 (14 months) number of isolates *	01 July 1995 - 01 July 1998 (36 months) number of isolates *
<i>Clostridium perfringens</i>	3	9
Other <i>Clostridium</i> species	2	9 †
<i>Propionibacterium</i> species	0	7 ‡
<i>Eubacterium</i> species	0	1
<i>Bifidobacterium</i> species	0	1
<i>Actinomyces</i> species	0	4 §
<i>Peptostreptococcus</i> species	4 ¶	12 **
Anaerobic gram positive cocci ††	2	0

* Excludes duplicate patient isolates. † Includes *Cl. septicum*, *Cl. difficile*, *Cl. cadaveris*, *Cl. bifermentans* and *Cl. sporogenes*. ‡ Includes *P. acnes*. § Includes *A. israelii* and *A. odontolyticus*. ¶ Includes *P. magnus* and *P. asaccharolyticus*. ** Includes *P. magnus*, *P. tetradius*, *P. micros* and *P. asaccharolyticus*. †† Not speciated further.

Table 8. Analysis of Positive Anaerobic Cultures by Specimen Type pre and post implementation of methodology changes

Organism Group	Blood Culture Specimens	Other Specimens*
<i>Bacteroides fragilis</i> group organisms †	18 (3)‡	15 (11)
Other gram negative anaerobes §	3	12 (2)
<i>Clostridium</i> species ¶	7 (1)	11 (4)
Other gram positive anaerobes **	3	22 (6)

Includes tissues, aspirates, fluids and abscess swabs. † Includes *Bacteroides* isolates unable to be speciated further. ‡ Numbers of isolates () pre implementation of methodology changes. § Includes *Fusobacterium*, *Prevotella* and *Porphyromonas* species and *Bacteroides ureolyticus* group. ¶ Includes *Clostridium perfringens*.

** Includes *Propionibacterium*, *Eubacterium*, *Bifidobacterium*, *Actinomyces* and *Peptostreptococcus* species and anaerobic gram positive cocci unable to be speciated further.

Table 8 shows analysis of the positive anaerobic cultures by specimen type. The number of anaerobes recovered from blood culture samples has increased from 4 (15%) to 31 (34%). The introduction of specimen collection criteria and the use of ATM has had no influence on this increase as procedures for inoculation and transport of blood culture specimens has not been changed. Therefore, this improvement is most likely due to the use of PRAS isolation media and an improved anaerobic incubation system. The numbers and types of anaerobes isolated from the non-blood culture specimens illustrates the impact that the combined use of a strict specimen collection and transport criteria, including the use of ATM, the changes in primary isolation media and the incubation system, have had on the anaerobe isolation rate.

Discussion

Collection and transport of specimens

Aspirated fluid, obtained by using a needle and syringe, is the best specimen for the recovery of anaerobes (1-3). It provides a greater volume of specimen and minimises contamination with surrounding microbial flora. Tissue specimens are also suitable specimens for anaerobic culture (3). A swab may be accepted for anaerobic culture only when collection by aspiration or biopsy is not feasible. Anaerobic swabs are poor specimens for several reasons including small volume of specimen, greater chance of contamination with normal flora and because many organisms may remain attached to the fibers of the swab (2,3).

Specimens must be protected from the harmful effects of oxygen until they can be cultured. It has been shown that the use of anaerobic transport systems increases both the quantity and variety of anaerobes isolated (3).

Primary isolation media

The four quality control strains used in this evaluation demonstrate that better results can be obtained with the use of PRAS primary isolation media. It has been well documented that unless freshly prepared media i.e. 24 - 48 hours old is available, PRAS should be used for the primary isolation of anaerobes (5,17). Media that have been exposed to oxygen contain oxidised products that may delay or inhibit the growth of many anaerobes (17). Note that plate media used for subsequent subculturing do not need to be reduced (5).

All anaerobic cultures should be supplemented with a broth enrichment culture. Thioglycolate broth supplemented with vitamin K, haemin and sodium bicarbonate, and cooked meat medium supplemented with glucose are excellent mediums for the primary growth and maintenance of anaerobic organisms (4,17). The broth serves as a back-up source of clinical material in case of jar failure or when growth is inhibited due to antibacterial factors e.g. polymorphonuclear leucocytes or as an enrichment for small numbers of bacteria in the specimen (5,17). The broth culture should never be relied upon exclusively for the isolation of anaerobes as they can be overgrown or inhibited by metabolic products or acids produced from more rapidly growing facultative organisms (17).

Incubation system

The surface growth of anaerobes requires an oxygen-free atmosphere. It is important that cultures are not exposed to oxygen until after 48 hours incubation, since anaerobes are most sensitive to oxygen during their log phase of growth (6).

The AnaeroGen™ anaerobic jar incubation system (Oxoid) chosen for use has been shown to perform reliably in its ability to support the growth of a wide variety of anaerobes (9). It is able to reduce the oxygen level in the jar below 1% within 30 minutes of set up. A carbon dioxide level between 9 and 13 % is also generated. This is important,

as many anaerobes require CO₂ for growth (1).

The AnaeroGen™ system is easy to set up and maintain. The anaerobic atmosphere generating system does not include a catalyst that requires regular maintenance and the anaerobic jars are not difficult to secure and seal.

Anaerobic biochemical identification systems

The increase in number and types of anaerobes recovered raised concerns regarding the ability of the in-use VITEK anaerobe identification system to accurately identify these isolates. After testing the VITEK ANI card in parallel with the RAPID ID32A system against a selection of quality control strains the decision was made to continue with the VITEK ANI identification card. This system is easy to setup, read and inoculate and performed well in the identification of the most common, clinically encountered groups of anaerobes.

This evaluation emphasised the importance of using the information obtained from the primary identification plates i.e. gram reaction and colonial morphology when identifying anaerobes, particularly with the incorrect identification results obtained from both of the systems tested. Also, the source of the specimen must be given consideration when processing a positive culture from a clinical specimen and evaluating the identification results.

Sensitivity testing

Anaerobic sensitivity testing does not need to be performed on all clinical isolates. Isolates from the following infections must be considered for susceptibility testing - refractory or recurrent bacteremia, central nervous systems infections, endocarditis, osteomyelitis, joint infection, prosthetic device infection, an organism isolated from any normally sterile site and any infection not responsive to empiric therapy (10).

The M.I.C. results for each anaerobe tested can be collated at regular intervals to produce cumulative sensitivity data. This information can be used to validate empiric therapy recommendations and to monitor changes in patterns of susceptibility over time. Adequate numbers of strains (at least 10 of each species) must be tested if inferences are to be made regarding a particular group or species (10).

The introduction of the Etest (AB Biodisk) method has enabled the performance of susceptibility testing of individual patient isolates against a few selected antimicrobials with a minimum amount of preparation, set-up and result interpretation time. Inoculum preparation, plate inoculation and application of test strips is quick and easy. Determination of the M.I.C. endpoint is clear and reproducible. This is an important feature of the methodology as determination of the endpoint is one of the greatest sources of error in interpretation of anaerobic susceptibility test results (10,12).

An M.I.C. cannot be reliably determined unless adequate growth of the test isolate has been achieved. The Etest method is performed on agar so that not only can adequate growth of an isolate be visually assessed on the surface of the plate but also contamination of the test inoculum, or mixed cultures, can be easily seen.

Conclusions

This review of the anaerobic microbiology area of the Health Waikato laboratory has resulted in the development of new protocols for the processing of specimens. Strict collection and transport criteria for every specimen requesting an anaerobic culture have been implemented. Specimens are processed in a timely fashion upon receipt in the laboratory, cultured onto PRAS media and incubated under carefully monitored anaerobic conditions. The VITEK ANI card is used to identify clinically significant isolates and sensitivity testing to a range of antimicrobials can be performed using the Etest methodology. The impact of these changes can be seen in the increase in the anaerobe isolation

rate from 2.5% to 37%.

These changes in methodology have achieved a more practical approach to anaerobic microbiology, whereby anaerobes can be reliably isolated and identified and sensitivity testing can be performed when required.

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TH Pullar Memorial Address

Controlling Change

Paul McLeod
Laboratory, Nelson Hospital

The theme for this conference is the motivation for change. For at least the last 15 years I have been attending these annual meetings and without doubt the subject of change has been a very common discussion point. I recall in years gone by this topic being addressed as "getting ready" for change. This was then followed with "coping" with change and now here I am talking about "motivation" to change. If you feel a little confused then I'm sure that you are not alone!

Change is a natural process. There is nothing special about it rather than to say, if as human beings we didn't go through it we would surely still be swinging through the trees. (No doubt an attractive alternative for some!) It is the ability of the human being to meet the challenges of change that sets us apart from other creatures. Sure, there are lots of fascinating examples of how other creatures have changed to deal with different environments, but it is our capacity to deal with a multiplicity of complex changes that sets us apart.

Richard Attenborough, a famous story teller of nature, has spent his life recording on film the wonders of animal and bird life behavior. We marvel at how these simple creatures have developed and adapted to survival in an albeit, slowly changing environment. Yet we do not give more than a moments thought to the skills that the human being has developed. Imagine for a moment, a room full of dogs, cats, chooks, horses, cows, etcetera, watching spellbound a television documentary on homosapiens. Their mouths would drop open as they see how this amazing animal has built nests in which to sleep and work. Then they would watch in awe as they witness what is obviously an organized past-time of competition: 15 creatures all dress the same as do an opposing 15 and then under the control of another dressed differently again, they all run around on a marked out patch of grass chasing an egg shaped object. And what makes this event even more special is that often thousands and thousands of other homosapiens gather around the grass patch to watch and shout. Other television programmes follow showing this amazing creature sliding down mountainsides on long planks, skillfully hitting a tiny white ball into holes with flags sticking out, riding waves, bicycles and even horses! Such a television series to our earthly animals would be simply stunning!

From the moment we completed that very short trip from our mothers womb, we all commenced a process of change which will continue on until the day we die. In the initial stages the changes we made are common to most life forms. We learnt to breath, suckle and cry. As the years go on, we move from involuntary change requirements to self controlled forms of change. The motivation for change gradually becomes an individual drive or force. We all have differing levels of motivation and next month in Sydney we will see thousands of homosapiens all of whom have in common, an incredible level of motivation for perfection in whatever sport they represent. In business we see similar motivation in the form of financial success, and in the arts, we watch and listen to the performers in wonderment.

As we travel on through our journeys, the aging process itself sometimes motivates us to change. We replace crumbling teeth, worn joints and various body organs. We correct failing sight and hearing.

Sometimes we go into prevention mode by swallowing copious quantities of vitamin supplements and health foods, or going to gymnasi-ums. But surely, if God wanted us to touch our toes, he would have put them on our knees.

So what is the point I am trying to make? There is nothing special about change itself. It is the motivation to develop and control it that is the variable. It is this area of self control and management of change that I would like to expand on in regard to the issues of our profession.

To get some feel for where medical laboratory science is now, we need to look back from where we have come. I can personally go back as far as 1967 when I started my training. No doubt there are some here who go back even further but looking at this audience I see many who have a shorter history. In the 1960's there were many (now considered quaint) downright dangerous practices. Mouth pipetting was the main means of measuring liquids. A mouthful of reagent was looked upon as something of amusement as the unfortunate victim raced to the sink to spit out the offending fluid and place their lips around the swan necked tap. From memory, Nessler's reagent used for urea estimation gave your teeth a particularly furry feel for a day or so. Eating and smoking in the lab was fairly common practice, but one of the highlights of my memories was the time when the Van Slyke apparatus fell over spilling about a half a litre of mercury over the floor. The fun in scooping that up still remains vivid in my mind. Being the lab junior, I was given the task of cleaning the mercury. This was achieved by placing a towel in a bowl. The mercury was poured onto the towel which was then gathered into a bundle and gently wrung by hand as if you were wringing water out of it. The mercury as it passed through the toweling left the dirt behind. All rather ingenious really. Blood was often collected by capillary sample by directly sucking it into a Sahli pipette via a piece of rubber tubing. The same process occurred in Haematology or Biochemistry from the tubes of samples arriving in the department. Chemical stores were the heart of the lab. Now with modern-day analysers and reagent kits, such rooms are considered an occupational hazard and need to be removed. Autoclaves also are quickly becoming a relic of days gone by as culture media is purchased ready to go. Counting chambers are to be found only on occasions in Microbiology. Mouth pipettes have gone along with the glass test tubes. The gurgling pipette washers are also redundant only to be replaced with the noises of the multichannelled analysers. The crowded benches of racks of test tubes and spectrophotometers have disappeared. The shelves crammed with "in use" reagents now stand largely empty or replaced with small cardboard boxes of reagent kits. If one was to become completely sentimental, it could be said that also missing today is the soul of the good old days when our profession was a true science, still blissfully unaware of the impending day of Standard Operating Procedure manuals, auditors and QA programmes!

I guess that in time, todays scientists will too have their happy memories of their early days in the lab. I recall one incident when one day arriving at work a technologist noticed a small fire burning in the bottom of a lift well that was being refitted. The workman had gone off

somewhere and thinking nothing of it, the technologist poured a beaker of water on to it and that was the end of that. A few hours later, this incident was being discussed at the bench. Someone recalled a company policy of reporting all fires and doing the correct thing phoned the hospital fire officer. Now this person had a very strong military background and in usual fashion responded to the call without waiting to hear of the conclusion. A few minutes later we could hear the distant but approaching sound of the siren. It so happened the access to the lab was down a steep narrow alleyway over the top of which was located a link corridor. The gap between the ramp and the corridor was unfortunately slightly less than the height of the fire engine and its ladders on the top. The resulting noise and commotion still remain vividly in my mind, but the attempt to explain to the rather agitated firemen that the fire had been extinguished some hours earlier was even more interesting. The same hospital fire officer also had the dual role of pest control officer. This rather unfortunate chap just seemed to lend himself to being open season for ridicule. Thrill-seekers such as lab staff and house surgeons had endless hours of fun with him. On one occasion he received a late night call from a house surgeon with the report that the main hospital corridor was being invaded with large mites. Could he please come in immediately and deal with the crisis. On arrival he was greeted with about two dozen crayfish aimlessly crawling up and down the corridor. The house surgeons had returned from a very successful days diving at d'Urville Island and after a few congratulatory beers had conjured up this dastardly act.

If you are sitting there thinking that labs have now reached their final destination in regard to change then think again. The extent or degree of change that has occurred over the last 10 years- horrendous as it has been will all happen again but within 5 or even less years. The pace is constantly picking up. Take for example Point Of Care (POC) testing. If you think that we have dealt with this issue you are very wrong. Just around the corner is a developing technology that will revolutionize how we and other health professionals will do our jobs. Already developed is noninvasive glucose and Haemoglobin estimations. There are available hand-held analysers capable of computer interfacing for data transfer which are multifunctional, simple to operate and accurate. These are yet to be fully embraced by medical and nursing staff and we still have an opportunity to establish ourselves as the source of reference in their use. There are numerous issues surrounding POC testing including selection of equipment, training, maintenance, industrial issues, data capture, ownership of results, quality assurance and costs.

We now have to motivate ourselves to change our ingrained beliefs, ideals and principles about POC testing if we are to ensure the best possible outcome for the patient. We could be like the chap (whose name escapes me) who stood on the beach and demanded the incoming tide to stop. On the other hand, we could pack up our towels and umbrella and move up the beach and watch the developments from the advantaged heights of the sandhills. We have no choice because to refuse to move will see all options lost.

So what do we do? Most of us would have several arguments as to why POC testing is unsatisfactory. However, to me the key issue is, what is best for the patient. Is it crucial to the diabetic that their glucose be measured to two decimal places in the lab or immediately in the Emergency Department within an acceptable margin of error? Does it really matter if a bloodgas analyser is operated by a trained but nonlaboratory person? The key issue is patient safety. It is my belief that POC testing is not a threat to the future of our profession. In fact, I believe that it will grow to supplement what we do. If you stop for a moment and think the logic through you will see what I mean. POC testing will be self-limiting due to its cost, "one off" nature in procedure and its relevance to any particular situation. Just consider for a moment what a hospital ward or GP's office would look like if all lab

procedures had to be performed by the bedside or in the office. How would they deal with the data management, equipment maintenance, staff training, specimen storage, IANZ audits and QC programmes. It would very soon be concluded by management that there were huge management risks involved, along with lengthy delays in the one off testing regimes plus very significant costs. Soon nurses would be complaining of not doing what they were trained to do and doctors surgeries would be taking on the appearance of mini laboratories. The conclusion would be that all the issues would be addressed by centralizing the lab services to be staffed by people qualified to do the job and ensure public safety. However, in saying this, I also believe that there is a rightful and proper role for POC testing and in most cases this will be because it is best for the care of the patient.

I recall some years ago listening to a presentation in Australia by an Intensivist who worked in a major emergency department. He had a differing view again about POC testing. He saw little or no need in his area of expertise for such a service. He said that he could not recall a single occasion where he needed a lab result that quickly. He went on to say that he would never act on a single lab result in isolation as he was in the business of monitoring trends. He went as far to say that any doctor who acted on one isolated piece of laboratory information should not be practicing medicine.

The Medical Laboratory Technologists Board also sees POC testing as an issue. The Boards focus is patient safety but unfortunately under its present Regulations can only apply its control to what happens within the walls of a medical laboratory. POC testing by its nature and definition occurs outside the doors of the lab. Such restrictions simply reflect the age of the Regulations under which the board has to operate. Hopefully, the new legislation on the horizon will allow the Board to ensure the safety of all the public regardless of where the lab procedures occur and by who performs them.

POC testing is an issue for all health professionals and it has brought about change within both hospital and community laboratories. I would now like to comment on another change that is still evolving and effecting all laboratories and that is the ownership of them. As hospital managers have struggled with increasing demand and decreasing budgets, reviews have occurred from one end of the country to the other on what their core services are. Some hospitals have retained their clinical functions but shed their support services such as food, laundry, cleaners and ground maintenance. These were sold off to the private providers where, I guess, it has proved to be a successful decision. Has anyone challenged this assumption? Are there any hospital managers who are looking to see if they could again run these services more efficiently than the private sector? Many of the private providers have now gone (away or under) and in some instances the remaining providers are now amalgamating thereby reducing the market competition which originally gave the hospitals value for money. The potential for market capture is high and many of the hospitals who abandoned their laundries and kitchens have little option but to stay with their private providers who no doubt are starting to see the power of their bargaining position.

Some other hospitals crossed the boundary lines and included laboratory services as non core. In some instances these labs have been closed with tests performed offsite. Other labs were sold off and private providers moved on site to the existing lab. In my opinion, the jury is still out in regard to whether or not these decisions were the correct. In the community labs, it would be fair to say that this sector has been turned on its head (several times) during the last decade. The owner operator styled labs of the 1980's are now predominantly owned by national or international companies. Buy-ups, sell outs, amalgamations, lab wars, you name it, it has happened. It has been the 'corner diary versus the supermarket' story all over again with centralization

and job redundancy fallout.

The business approach to health by recent governments has exacerbated this environment which has resulted in the fragmentation of our professional brotherhood, and these changes are still taking their toll on our profession as we struggle to adapt. Way back in the mid 1980's many of you will recall the industrial action our profession became involved in. To briefly recap, after a period of eye-watering inflation, the government was digging its toes in over salary increases. They weakened momentarily and gave substantial increases to doctors and nurses but then slammed the door shut in our faces. I raise this issue because it has some relevance to what I'm talking about. During this time I recall, the Institute negotiations team having incredible difficulty in explaining our role in the health sector and what it actually was that we did. The bureaucrats in Wellington had little or no understanding of the issues and, quite frankly it would have been easier to make a jelly bounce than it was to get this through to them. This same sort of problem was still present in some governmental departments as recently as two or three years ago. On that occasion, the Ministry of Health was picking up on a report written several years earlier by the Commerce Commission that medical laboratory technologists should not be a registered profession as they present no direct risk to the patient. Deregulation was the catch cry of the government of the day and here was a group (along with a few others) who could be dealt to. The basis of the deregulation argument was that it is the doctor who acts on the lab results and therefore it is he or she who presents the risk to the patient. We argued that the doctor acted on lab results in the good faith that the result was accurate. Fine they said, but surely the doctor would recognize an aberrant result ! You can see the level of thinking here can't you ! We used the analogy of an air traffic controller giving advice to a pilot but even this failed to impress. This reminds me of a story, and I hasten to give in advance an apology to both the Irish and my pathetic attempt at their accent.

"This is Shamrock Air to tower, do you read?"

" This is Galway Tower to Shamrock Air- who am I speaking to?"

" This is Paddy- is that you Shawn?"

" Aye, it is that Paddy, how can I help?"

" Can you give me my height and position Shawn as I'm a bit lost at present. "

" Certainly Paddy, but I don't know if it will help much. You're 6 foot 2 and sitting up the front! "

Hopefully now after what has happened at Gisborne, there is an appreciation of the importance of lab data and its accuracy. This tragic situation will hopefully serve the purpose of demonstrating our argument. Several years ago I learnt a valuable lesson. You may recall Richard Prebble as a Labour MP in the House exposing a scandal at the Auckland Blood Transfusion Centre. He had been informed that outdated kits were being used for testing HIV on blood donations. As it transpired, it apparently was a situation where one of the reagent components had outdated by a week or two, however the accompanying calibrations and controls all indicated that the test results were reliable. This practice was happening in an environment where the same politicians were cutting health budgets and insisting that all health professions tighten their belts! The delight that the politicians have in exposing such practices (especially when in opposition) is aggravating to say the least. Such practices were (and maybe still are) commonplace as we genuinely wanted to achieve maximum economy without endangering public safety. But the mention of outdated reagents and HIV by a politician and you are on a hiding to nothing. The lesson I took: I altered my philosophy and to hell with the cost.

The public expectations of laboratory services have been brought to light courtesy of the Gisborne experience. It is obvious to me that their expectations in some laboratory procedures are unrealistic. It is made

confusing by the fact that where some procedures are objectively accurate to two or three decimal places, next door in Microbiology or Histology, results of a subjective nature are churned out on a daily basis. The quality of their results are heavily dependent on the quality of the sample received and this fact is one over which there is little or no control. Just how these variances of reporting from a single lab can be adequately explained to the public is something that I do not have the answer to. Hopefully the result from the Gisborne enquiry may address this issue.

Public expectations and political pressure are issues we have to accept and deal with. A good example is the new requirement for the New Zealand Blood Transfusion Service to filter out white cells from blood donations. As I understand it, this is a rather expensive procedure but will amongst other things reduce or remove the likelihood of transmitting CJD via a transfusion. The public and political fear of transmitted disease via blood transfusion is high, but as I understand it, nowhere in the world, after millions of transfusions is there any evidence of such contamination and transmission occurring.

So now with political and public awareness of the laboratory services sharply focused on us, the good old days of the backroom boys and girls in the lab have gone. We now welcome in a service which has all procedures documented and audited. We can no longer stray from the path of the procedures manuals and if you wish to pursue a scientific career which has flexibility, innovation or research, a medical laboratory is probably not the place for you. With the rate of change occurring at an increasing pace and the technology changing even faster, I believe we can congratulate ourselves on our forward thinking in changing our training requirements some years ago. The old apprenticeship training we had was fine when technology was relatively stable in its development. However, the rapid change in technology today would leave that method of training exposed. A comment often made is that the graduates today are not as productive on the bench as the newly qualified apprentices were. That is no doubt the case in many situations but is not surprising. The true test of our training system will come some years later with graduates demonstrating their ability to deal with the amazing technological changes that are occurring. Their base knowledge will be far superior to their apprenticeship equivalents. Our future scientists require an in-depth knowledge of scientific principles as well as technique and I believe that is what the BMLS provides.

I was looking back through past Thos Pullar Memorial addresses, mainly to determine what I had let myself in for ! It is as interesting, as coincidental, that the first such address was given in 1967, the year I commenced my training. The presenter was Dr Gunz who spoke on the subject of cytogenetics. Here 33 years on I confess that I still have trouble with that subject and as for matching up all those chromosomes, yet alone detecting translocations etc - completely beyond me! However, what caught my eye in that journal issue was one of two articles written by Walter Wilson entitled "Thromboplastin Standardisation". He described the difficulties in obtaining a standardized thromboplastin so that the results obtained at various laboratories would be comparable. Due to lack of availability, labs used to make their own thromboplastin to determine the prothrombin time or PCT as it was known. The variable between each batch within the same lab was issue enough, but comparisons between other labs was another problem altogether! I recall an incident about this time when a brain was donated to the lab via the mortuary for processing to thromboplastin. In an old copy of Lynch is a method of how to go about the process.

"Thromboplastin is obtained from human brain. It may be made as follows: The meningeal membranes are removed and the brain is washed under tap water. The brain stem is usually discarded since it is a poor thromboplastin source. Portions of the brain are then emulsified

in a blender with acetone until the brain appears "flaky". Decant the supernatant acetone into water and repeat the emulsification until the water does not become milky when the acetone is added, indicating that all acetone-soluble lipids have been removed. Spread the residual brain material on dry paper towels and allow to dry at room temperature. The dried material should be powdery and pale tan, and it may be stored at 4C in airtight containers; it keeps indefinitely. For use, 0.5gm. of dried brain powder is suspended in 10ml saline and incubated at 37 C for 15 minutes with gently stirring, and then the coarse particles are allowed to settle out. Control one-stage prothrombin times are performed to obtain the range of normal values, which should fall in the range of 11- 14 seconds."

The main problem for us was obtaining an homogeniser, a rather modern day appliance at the time. One of the trainees (wanting to impress no doubt) offered her mother's homogeniser. This was fine until her mum later discovered what it had been used for - we couldn't understand what all the fuss was about! The hospital purchased a replacement homogeniser for the mother, and the lab obtained the "donated" second hand one for future use. I tell this story just to highlight how far we have come in the last decades. We can laugh at these situations but I simply wish to remind you all that in another twenty years or so there will be other scientists holding their sides and roaring with laughter at the practices we carry out today. Today will be the good old days by then!

Into the future, we will rely more and more on our Institute and fellow scientists to help us manage the changes that lie before us. Continuing competency in the way we practice will become increasingly important. The public, via the Board will insist that such a demonstration will be required for you to practice. There is currently being drafted a new Bill to replace all existing Acts and Regulations. The very title of the Bill reflects this. It is entitled the "Health Professionals Competency Assurance Bill ". This will be an umbrella Act under which all health professions, including the Board, develop their own Regulations. However, the over riding principles include continuing competency to practice. This Act will set the scene for many year ahead. It is there for public protection and not for the health professionals. The Board is the organisation charged with ensuring that the legislation as prescribed is carried out. Interestingly enough though it is a self supporting organisation totally funded by the profession. So here we have political demand for public safety being funded by the practicing health professionals who in turn could be disciplined if they are found to be wanting under any of the regulations.

It has been an honor for me to give this address today. Unlike many before me who have known Thos Pullar, I can only recognize an often published photograph of the man but apart from that, rely on other's memories. Obviously, he was a huge influence in his day. His commitment to improving the lot of technologists and education is beyond question. Dr Cunz in the first address stated: "Dr Pullar was never happier than when he was sitting with a colleague or a technologist at a laboratory bench. Clinical pathology was his love, and he was very ready to learn. New methods and glimpses of new horizons fascinated him"

Well those new horizons are still there. I wonder what he would make of today's laboratory and its future.

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Minutes of the 56th Annual General Meeting of the New Zealand Institute of Medical Laboratory Science (Inc) held at the Rotorua Convention Centre on Thursday 17th August 2000

Chairman

The President (Mrs A Paterson) presided over the attendance of approximately 46 members.

Apologies

Nil.

Proxies

A list of 2 proxies were read by the Secretary.

Minutes

Motion:

Moved J Melvin, seconded W Dellow

That the Minutes of the 55th Annual General Meeting held on Wednesday 25th August 1999 be taken as read and accepted as a true and correct record.

Carried

Business arising

Nil.

Remits

Motion:

Moved T Rollinson, seconded S Gainsford

That Policy Decision Number 4 be reaffirmed

Policy Decision No 4 (1991): That the Code of Ethics as circulated to all members be adopted by the New Zealand Institute of Medical Laboratory Science (Inc).

Carried

Motion:

Moved T Rollinson, seconded C Kendrick

THAT Policy Decision Number 6 be reaffirmed

Policy Decision No 6 (1979): That the Council must be informed in advance of national workshops, seminars or similar gatherings which are being conducted under the aegis of NZIMLS organisations.

Carried

Moved T Rollinson, seconded D Dawson

THAT the subscription rates for membership be adjusted from 15th April 2001 to be:

Members \$112.00

Associates \$56.00

Non Practising \$56.00

Carried

President's report

Motion:

Moved A Paterson, seconded D Reilly

That the President's Report be received.

Carried

Annual report

Motion:

Moved L Milligan, seconded S Melvin

That the Annual Report be received and adopted.

Carried

S Gainsford acknowledged the role that the MLTB played in starting off the MOLS programme and in particular D Reilly's involvement in getting it up and running. S Gainsford advised that she had extensive meetings with other medical professional groups and noted the MOLS/CPD programmes are ahead of other groups.

Financial report

Moved

T Rollinson, seconded D Dawson

That the Financial Report be received and adopted.

Carried

Discussion:

S Gainsford questioned why accommodation had increased from \$5,000 to \$11,000? This was due to the increase of 3 to 4 Council meetings and the cost of hosting the President and Vice President of AIMS at the South Pacific Congress.

W Dellow noted that with the increase in Fellowship dissertations being published in the Journal, there will be an increase in cost of producing the Journal. Council is investigating ways that the costs of the Journal can remain within the present costs.

T Rollinson acknowledged and congratulated P Reilly for the tremendous contributing she makes to the NZIMLS and Journal in her role as Advertising Manager.

Election of officers

The following members of Council were elected unopposed:

President	A Paterson
Vice President	L Milligan
Secretary/Treasurer	T Rollinson
Region 1 Representative	S Benson
Region 3 Representative	C Kendrick
Region 4 Representative	G Moore
Region 5 Representative	S Melvin

Awards

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

Life Membership of the NZIMLS

Michael Lynch PPTC, Wellington

Qualified Technical Assistant Awards

Clinical Biochemistry	Andrew Hollingsworth, Diagnostic Laboratory
Medical Cytology	Janiene Gilliland, Medlab Central
Haematology	Manisha Morar, Wellington Medical Laboratory
Histology	Tracey Gunn, Medlab Timaru

Immunology	Karen Heatley, Canterbury Health Laboratories
Microbiology	Terry Mather, Diagnostic Medlab Donna Doreen, Medlab Central
Mortuary	
Hygiene & Technique	David Dawson, Lakeland Health
Transfusion Science	Diane Garaway, Middlemore Hospital Transfusion Science
Blood Products	Irene Rankin, Lakeland Health

Honoraria

Motion:

Moved T Rollinson, seconded S Gainsford

That no honoraria be paid.

Carried

Auditor

Motion:

Moved T Rollinson, seconded P McLeod

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

Carried

General Business

Acknowledgement of book

D Reilly was asked by Warren Johns of Auckland to mention his book which he has recently published "Basic Laboratory Equipment" at a cost of \$9.50.

2001 NZIMLS conference

Raewyn Bluck advised that this will be held in Auckland and organised by the South Auckland Healthcare group at Middlemore Hospital.

Are unable to have this conference in August and therefore requested that an exemption to hold the AGM a week after the requirement of 7th September be considered.

Motion:

Moved R Bluck, seconded P McLeod

That the 2001 NZIMLS ASM be held 12th-15th September 2001 and proposed that the membership agree to holding the AGM on 13th September 2001 which is a week past the requirement date.

Carried.

Deaths

• Brian Millar, Auckland acknowledged that Bill Wiggle had passed away recently.

• P McLeod, Nelson acknowledged the death of Alan Harper.

Website

C Kendrick was thanked for the work on the website, which included information of the CPD programme and links to other organisations.

C Kendrick requested that he be advised of any dates for scientific events and programmes.

IAMLT

D Dixon-Mciver questioned the NZIMLS membership of the IAMLT. Noted that the Australian Institute had recently resigned their membership again and questioned what the NZIMLS was getting out of retaining their membership of the International body.

A Paterson advised that this topic had been discussed every year since

her time on Council (1989). Noted that the NZIMLS Executive would be meeting with AIMS representatives and would discuss this issue with them.

D Reilly acknowledged that he had just finished a four year term on the IAMLT Council, noted that working at an international level was very difficult and outlined reasons for continuing with membership of this organisation.

- IAMLT is the resource that the WHO uses when it has laboratory questions. WHO moves very slowly and therefore the IAMLT struggles to make progress.
- IAMLT supports countries setting up associations.
- Have increased the number of awards that they offer. Noted that very few people apply for these awards.
- IAMLT runs a congress every two years.

Acknowledged that the IAMLT is in financial difficulty and need to cut costs.

Questioned if the UK Institute had resigned their membership as well. Felt that there are little benefits being directed to the Pacific area.

D Reilly did take a proposal from M Lynch to the IAMLT, that we have a regional group for the Pacific, but this was not supported. Noted that Fiji is no longer a member of the IAMLT.

Noted that as D Reilly is no longer involved, there is no representation from this region. All Council members are from the northern hemisphere.

S Gainsford noted that she has been opposed to this membership for sometime and in all this time the IAMLT have not been able to 'put their house in order'.

Motion:

Moved D Dixon-Mciver, seconded S Gainsford

That the NZIMLS withdraw from the IAMLT.

Amendment

Moved M Lynch

That Council review this matter after they have discussed it with the Australian executive team.

D Reilly spoke against this motion. As the subscription is not due again until 31st March next year, that the NZIMLS write to the IAMLT asking what their plans are and advising them of the concerns of this meeting. Suggested to hold making a decision until the 2001 AGM. The above motion was put to the vote and the result was 8 for the motion and 28 against. Two members abstained. Therefore the motion was lost.

A Bunker questioned if there were any risks of the NZIMLS remaining members.

Council advised that they had sought a legal opinion looking at the NZIMLS liability for any debts that the IAMLT may have. The Solicitors best advice was that the NZIMLS would not be liable.

Motion:

Moved P McLeod, seconded D Dixon-Mciver

That the Council refrain from paying the next subscription until this is discussed at the 2001 AGM.

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Carried. One member abstained from voting.

This issue is to be commented on through the Council News and should be discussed with colleagues in the laboratories.

Phlebotomy group

A Bunker believes that there needs to be standards for Phlebotomy. These people are at the 'point of testing' care and need to be involved in this.

Wants to look at a qualification for these people and they would like to be part of the NZIMLS membership.

Linda Manuel, IANZ acknowledged that these people are accredited as a separate discipline.

Also noted that someone in Christchurch is involved with a NZQA training and qualification.

Further comments can be made through the Council News on this issue.

Gisborne issue

P McLeod, Chairman of the MLTB was questioned on the issue relating to ethics in the light of the issue in Gisborne.

P McLeod was unable to comment. Noted that the MLTB is there only to protect the public against a person, but not against a laboratory.

Venue for the year 2002 Annual Scientific Meeting

No venue was confirmed. Council will follow-up.

Meeting closed at 5.26pm

NZIMLS President's report

At the turn of the millennium, 55 years after our organisation was formed, we could be considered a success. We have survived, we have made progress, and strive often successfully to meet the myriad of requirements of the day.

Today's success is built on the hard work of many before and yet is as fragile as a flower, so requires all of us to continue to nurture it to ensure continued success and growth. Criticism is good, criticism without constructive options offered is not.

Financially, the systems put in place over the past 4 years, predominantly under the stewardship of my predecessor, Shirley Gainsford, now allow Council to more closely monitor expenditure and income. This will continue to be modified as necessary.

We now have a contract with our Executive Officer and her firm, Executive Events, which protects both parties more than an "employment" of Fran would. Through this our Executive Office continues to be accessible 40 hrs/week to service the various needs of our members. Non members must pay.

Membership has shown a small increase over the past year. The membership database system has been upgraded, but as with any computer program, it is only as good as data input, so please let our Executive Officer know if you change address etc. so that membership resources are not wasted.

The SIGs continue to provide excellent informal programs that complement the more formal coming together of the disciplines at our ASM. The SIGs are encouraged to utilise the appropriate assistance from our Executive Officer in managing their activities. All NZIMLS activities need to be fully auditable according to the laws of this land.

The window of opportunity for those holding a Specialist level of qualification to be exempt from Part I of Fellowship closed on 31st March 2000. In addition to those who took up this opportunity in the previous 3 years, an additional 34 members now challenge themselves (and the Fellowship Committee) to meet the requirements of Part II to gain Fellowship status through our professional body.

Increasingly the public and public watchdog organisations are focussing a critical eye on health services, including laboratory work. The HFA now requires all operating laboratories to be accredited, and very few have yet to gain that rank. Soon all laboratory practitioners will also require demonstrable evidence of competency and Continuing Education (CE).

Through the foresight of the MLTB, the pilot MOLS program ran from 1996 to 2000. Following extensive discussions between the MLTB and NZIMLS, and a subcommittee of Council and SIGs, it was determined that:

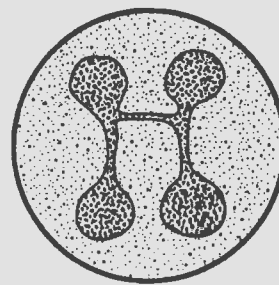
- the program would be administered by the NZIMLS
- modifications and extensions were required
- a data base on CE patterns was required
- that the program would only be open to financial members of the NZIMLS

The rationale for the last being clearly that so much of the program is to be administered via the NZIMLS Executive Officer that only members support through their membership fee. The Executive Officer will maintain records for and on behalf of members who submit the necessary documentation.

The Continuing Professional Development (CPD) subcommittee of the NZIMLS will be responsible for monitoring 10% of participants annually and an annual review of the components that make up the CPD program. At any time the MLTB will be able to audit both the program and its participants in its legally responsible role of public watchdog. It is likely that in the current health environment that the CPD program will become compulsory for all licensed practising medical laboratory scientists.

Council on your behalf will continue its voluntary efforts to administer our professional body, but I extend the invitation and responsibility to all members that this is a democratic organisation. Council welcomes constructive comment from the membership at any time.

Anne Paterson, President NZIMLS.



Journal Questionnaire

The New England Journal of Medicine, Vol 342, No. 17, April 27 2000, 1255-1265

Review - Medical Progress: Myelofibrosis with Myeloid Metaplasia A.Tefferi,M.D.

1. Myeloid metaplasia is always associated with bone marrow fibrosis. T/F
2. The terms "agnogenic myeloid metaplasia (AMM)" and idiopathic myelofibrosis are referring to the same condition. T/F
3. The trilineage proliferation seen in AMM originates in stem cells and is monoclonal. T/F
4. The finding of myelophthisis of the blood is pathognomonic for AMM. T/F
5. Cytogenetic studies show specific abnormalities accompany all cases of AMM. These include trisomy 8, 13q and 20q. T/F
6. Differentiating between AMM, myelodysplastic syndrome with myelofibrosis and acute myelofibrosis, is important as AMM has a better prognosis. T/F
7. The median age at diagnosis of AMM is 65 years and has been found to be more prevalent in males with a M:F ratio of 3:1. T/F
8. Patients with AMM are often anaemic. This is due to a number of factors including:
 - ineffective haemopoiesis
 - erythroid hypoplasia
 - the replacement of normal haemopoetic tissue
 - with collagen fibrosis
 - hypersplenismT/F
9. Complications of AMM include portal hypertension, splenic infarction, musculoskeletal and joint pain which is refractory to NSAID's, and lymphadenopathy. T/F
10. In patients with AMM the presence of any two of the following factors will severely reduce median survival: anaemia, leucopenia, leucocytosis, circulating blast cells, karyotype abnormalities, hypercatabolic states. T/F
11. What are the four main causes of death in patients with myeloid metaplasia?
12. What three drugs may be used to control leucocytosis in AMM?
13. What clinical variables indicate poor survival rates post splenectomy?
14. If splenic irradiation is ineffective then splenectomy is always the next treatment option. T/F
15. When anaemia or osteosclerosis are present the survival rate post allogenic stem cell transplantation is significantly reduced. T/F
16. Autologous haemopoetic stem cell transplants have shown promising results in early studies and are being promoted as a possible cure for the future. T/F
17. No antifibrotic or antiangiogenic drugs have shown conclusive evidence of clinical usefulness at this time. T/F

For the answers turn to page 00

For copies of this article please contact:

A. Rhodes
Haematology Dept
Diagnostic Medical Laboratory
Phone: (09) 571-4072
Fax: (09) 571-4057

Answers

1. False
2. True
3. True
4. False
5. False
6. True
7. False
8. True
9. True
10. True
11. Infection, thrombohaemorrhagic events, heart failure, leukaemic transformation.
12. Hydroxyurea, interferon alfa, cladribine.
13. Predominance of immature granulocytes or presence of microscopic infarcts in the removed spleen tissue.
14. False
15. True
16. False
17. True

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Please register me for the 2000 NICE Weekend

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Address for receipt:			
Phone:	Fax:	E-mail	

Paper or Poster: (Circle) Title:

A brief abstract of your presentation **MUST** accompany your registration form by 25th March 2000 .

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

Registration Fee	- \$ 250	\$
Or for NZIMLS members	- \$ 220	
Private Room Surcharge	- \$ 125	\$
I wish to share a room with		_____
Late Registration Fee (payable after 25 th March)	- \$ 50	\$
I enclose a cheque, made out to "NICE WEEKEND" for the amount of:		\$

Applications received after Friday 25th March 2000 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:

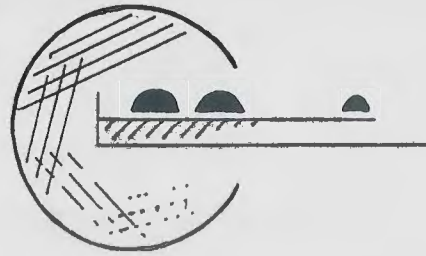
Please send form and cheque to Andrew Mills, New Zealand Blood Service – Waikato, P.O. Box 185, Hamilton. Note registration is limited to 50 participants and preference will be given to NZIMLS members .

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



This year sees a new team looking after the MSIG. Thanks to Sandie Newton, Jenny Dowling, and Janet Wilson for their efforts over the last few years.

Steve Soufflot (convenor)	Medlab Hamilton (07)8340780
Sue Earley	Starship Hospital
Jodie Cranfield	Starship Hospital
Linda Manuel	IANZ
Catherine Tocker	Waikato Hospital
Kay Stockman	Waikato Hospital
Tina Littlejohn	Medlab Central (Palm Nth)
Julie Vincent	Canterbury Health Lab.

Projects currently being worked on include a QTA syllabus review, and investigations into an e-mail network to allow simple communication between microbiology labs throughout NZ.

2001 MSIG Seminar - to be held in Hamilton on Saturday March 31st. Contacts are Catherine Tocker or Kay Stockman at Waikato Hospital.

Abstracts from the 2000 MSIG Seminar

A very successful seminar was held in Auckland with almost 150 participants and 20 presentations.

Incy Wincy

Kevin Drysdale - Nelson Diagnostic Laboratory

Sporothrix schenckii has a worldwide distribution. It is found in soil and on decaying vegetation. *S. schenckii* shows thermal dimorphism, ie at 30°C it grows as a mould, while at 37°C it grows as a yeast. The mould presents as a cream coloured, smooth colony, which turns brown in patches over time. Under the microscope, the conidiophores are at right angles to the hyphae and at the apex are clusters of conidia.

The yeast is a typical yellowish colony that shows budding formations microscopically. Looking back on the case, the two puncture marks on the patients legs were attributed to the violin spider, known to inhabit forestry mines where this patient had previously worked in Tanzania. The spider makes its nest in the milled timber and is known to transmit sporotrichosis.

An Unusual Isolate

Liz Frater - Capital Coast Health

An unusual, fastidious spiral-shaped gram negative bacillus was isolated from the blood of a patient (male, aged 40 yrs) who presented with cellulitis. The patient later tested positive for the Human Immunodeficiency Virus. Using the BACTEC 9000 System, six out of a total of nine blood culture sets taken grew the organism, taking four to six days.

The spiral organism which was difficult to see in the gram stain, proved difficult to grow on solid media, eventually growing on Charcoal media, Columbia Blood Agar and Wilkens Chalgren Agar at 37°C in anaerobic conditions. Although initially identified as a *Campylobacter species* (organism was oxidase and hippurate hydrolysis positive) it was subsequently thought to be a *Helicobacter species* and was forwarded to ESR for confirmation of identification by gene-sequencing methods.

A Cryptic CSF Case Study of a Patient with Lymphoma

Debra Maddocks - Medlab Central

An elderly patient presented with a 10 year history of low grade lymphoma. Over a 4-5 day period he suffered the following symptoms: photophobia, neck pain, headaches, and a lack of coordination. Consultants suspected central nervous system involvement.

A lumbar puncture was taken and results read: Microbiology: 155 WBC with 98% mononuclear cells. NOS in cytospin gram stain. Cytology: Extensive lymphoma cells noted by haematologists. These results indicated lymphoma in the central nervous system. 48 hours later there was an unexpected light growth of yeast on the culture plates. Confirmatory tests identified the organism as *Cryptococcus neoformans*.

The moral of the story is that even a classic CSF profile cannot be taken for granted - you may have a lurking *Cryptococcus*.

Campylobacter upsaliensis

Grace Lee - Diagnostic Medical Laboratory

In recent years, cases of human campylobacteriosis have increased tremendously and have achieved widespread and fairly rapid recognition. The most common species of *Campylobacter* are *Campylobacter jejuni* and *Campylobacter coli*. This study was about *Campylobacter upsaliensis*, a recently discovered organism that is slowly being recognised as an enteric human pathogen.

This study was done in May 1999 and I have decided to do a paper on this organism to see whether it can be recovered here in New Zealand. We processed 984 diarrhoeic faecal samples using modified CCDA (a commercially available *Campylobacter* selective medium containing cefoperazone (32 (g/mL) and amphotericin (10 (g/mL) in a blood free selective agar base), and CAT, a new selective medium for the isolation of thermophilic *Campylobacter* (blood free medium containing cefoperazone (8 (g/mL), amphotericin (10 (g/mL), and teicoplanin (4 (g/mL). Isolation rates were compared. If the organism grew on both plates it is presumed to be *C. coli* or *C. jejuni*, and if it grew only on CAT, the organism is further identified - presumably *C. upsaliensis*.

Of the 984 samples we collected and processed, 80 grew on both plates. There was no growth on CAT alone. I concluded that *Campylobacter upsaliensis* can not be recovered here in New Zealand

at the present time.

Primary Amoebic Meningoencephalitis caused by *Naegleria fowleri*

Matt Akehurst - Health Waikato

Naegleria fowleri is the main protozoan causing primary amoebic meningoencephalitis in humans. In New Zealand primary meningoencephalitis has mainly been implicated with swimming in hot pools. It enters the brain via the Cribriform plate through water entering the nasal cavity. Incubation period is usually 2-3 days; there is an abrupt onset of symptoms including fever, stiffness of the neck, fixation of the pupil, and nausea, thus mimicking bacterial meningitis. Differential diagnosis from bacterial meningitis can be achieved by heating a wet film and observing the characteristic movement of the amoebae. Amoebae can be mistaken for leucocytes in a counting chamber, therefore most cases of primary amoebic meningoencephalitis are diagnosed on post-mortem. It is recommended to treat with Amphotericin B, however the outcome of the disease is very poor with only about a 1% survival rate.

A Tale of Two Specimens

Marc Smith - Medical Laboratory Wellington

A 7 year old male, recently returned from Samoa, had been feverish for 3 weeks. One week previous, the patient had a urine specimen with $>10^8$ organisms/L of an *Acinetobacter* species. Two urine and one faeces specimens were received on the same day. One urine had $<10^7$ /L of mixed flora. The other urine had $>10^8$ /L of *Salmonella typhi*. Only one colony of *Salmonella typhi*, originally overlooked on the Hektoen plate, was isolated from the faeces. The *Salmonella typhi* was phage type E1a which is endemic in Samoa. Faster diagnosis could have been achieved by the taking of blood cultures earlier on, and you should never rely on one specimen to give the full picture.

***Burkholderia pseudomallei* in a Cystic Fibrosis Patient**

Denise Wheatley - Medlab Hamilton

Burkholderia pseudomallei is an environmental organism which is found primarily in tropical and subtropical areas. *B. pseudomallei* causes a life threatening disease called melioidosis.

In June of 1999, Medlab Hamilton isolated *B. pseudomallei* from a young cystic fibrosis (CF) patient who had recently moved from Darwin. No record of *B. pseudomallei* in CF patients was found in the literature. This child was not exhibiting symptoms of melioidosis. Colonisation most likely occurred before leaving Darwin. At that stage only one other CF patient had grown *B. pseudomallei*. That patient was treated very aggressively and remains colonised. In comparison, the Medlab patient was not treated and remains well to date.

Since then another CF patient in Darwin was diagnosed with *B. pseudomallei*. He was a close family friend of the Medlab patient. Was spread occurring between these patients? Both isolates were subtyped by pulsed-field gel electrophoresis and both yielded different results.

In Search of MRSA

Cathy Brehmer - Starship Hospital

The amount of Methicillin Resistant *Staphylococcus aureus* (MRSA) in New Zealand is steadily increasing and isolation procedures are becoming increasingly important. Mannitol Salt Agar (MSA) has been the standard medium for isolation of *Staphylococcus aureus* for many years. This is despite the fact that less than 100% of *Staphylococcus*

aureus ferment mannitol. In a recent Canadian study, only 94% of *Staphylococcus aureus* were positive for mannitol fermentation.

The Microbiology Laboratory at Starship Children's Hospital has recently been involved in investigations into an outbreak of MRSA in our Neonatal Intensive Care Unit. The strain of MRSA involved in this outbreak was found to be negative for mannitol fermentation, and gave atypical pink-to-salmon coloured colonies on MSA. This could lead to colonies of MRSA not being identified. It is therefore important that in investigations for MRSA, laboratories do not rely solely on a medium that does not give typical reactions 100% of the time. Laboratories should always include a non-selective medium when processing MRSA screening swabs.

Current Isolation Trends of *Bordetella pertussis*

Sandie Newton - Diagnostic Medlab

As everyone is aware we are currently in the throes of another *Bordetella pertussis* outbreak in New Zealand. Presented are some statistics for DML Auckland. January 1996 was the last outbreak peak with 28 culture positive cases. January and February 2000 had 55 and 58 isolates respectively, exactly 4 years since the last outbreak. Of these culture positives, almost half were in the 5-9 year age bracket whereas a comparison to NSW showed the 10-19 year bracket as having more isolates. Overseas studies show that in childhood infections, a significantly higher attack, morbidity and mortality rate occurs in girls. So far a breakdown of our statistics for 1999 and 2000 revealed 55% were males and 45% females.

Waikato QC Program Results

Kay Stockman - Health Waikato

The Waikato QC Program was set up six years ago for the disciplines of Biochemistry and Haematology, and in 1999 was expanded to include Microbiology. The aim of the program is to provide a basic quality control relevant to the daily work-flow of Microbiology laboratories, in particular the smaller laboratories.

A monthly dispatch of three questions and up to four specimens are sent for identification, sensitivity testing and interpretation. Results are reported anonymously in an educational summary. Results received during the first year of the Microbiology Program have been illustrative of the diversity in methodology and interpretation of microbiological testing in New Zealand. Some examples of these results are given.

***Neisseria gonorrhoeae*: Trends Sens and Tests**

Anne MacCarthy - STD Lab, A+ Laboratories

Auckland is a sentinel laboratory which monitors the sensitivity of *N. gonorrhoeae* on a regional basis, from both public and community laboratories in Auckland and from the Waikato Hospital. The antibiotics tested are Penicillin, Ciprofloxacin, Tetracycline, Ceftriaxone, and Spectinomycin. Results are sent to the Australian Gonococcal Surveillance Program, (AGSP) the regional representative of the World Health Organisation. MIC testing is performed by standardised AGSP method based on the agar plate incorporation technique, with the inclusion of six WHO control strains in each batch.

Penicillin resistance (>1.0 mg/ml), both plasmid and chromosomally mediated, has remained constant over the last ten years, ranging from 8-16%. Total resistance has always been above the 5% threshold, above which WHO does not consider penicillin to be effective in controlling gonorrhoea. Ciprofloxacin resistance (>1.0 mg/ml) has remained at $<2\%$, but in 1999 it surpassed the 2% threshold. This is

significant given that a similar trend of increasing resistance has been seen in other countries. In parts of Asia, resistance of over 50% can be found.

Our laboratory has noted a recent rise in the numbers of gonorrhoea that are sensitive to Vancomycin. On one occasion 10% of our isolates were in this category. The significance of this information needs to be addressed as Vancomycin is one of the antibiotics present in Thayer Martin media which is used by a number of laboratories as their sole selective media. Thayer Martin should only be used for sites where there is a large number of commensal flora present, eg throat and rectal sites. Routinely our laboratory uses fresh New York City Agar Auckland formula, which is the standard WHO recipe with no additives present, and a shelf life of a maximum of four weeks. (Available from Fort Richard. Code NYCA.)

Auckland STD section is currently auxotyping and serotyping their isolates to build up a profile of the gonorrhoea population in this region of the world. We have shown that the majority of the isolates fall into a small number of groups, but when the three profiles are combined (MIC, Auxotype, and Serotype), a large variety of types are present. This information is useful for special contract tracing, and comparing the isolates from a victim and the alleged abuser. These tests are very expensive and are only carried out in special circumstances.

The number of *N.gonorrhoeae* isolates in the Auckland regions have shown a steady rise since 1993. In particular the more recent upsurge of gonorrhoea both nationally and internationally has sparked interest at Ministry of Health levels. The continued monitoring of both sensitivity levels and numbers, continues to be very important as gonorrhoea is a marker for other sexually transmitted infections.

Sooner than Later

Ruby Yee - Hutt Valley Health

For earlier detection of Methicillin Resistant Staph Aureus (MRSA), we evaluated both the Denka Seiken MRSA Screen Agglutination Kit and Methicillin Aztreonam Mannitol Salt Agar (MAMSA).

The MRSA Screen Kit proved to be very useful in confirming MRSA isolates from both the sensitivity plates as well as directly from primary plates. We had one isolate which was oxacillin resistant and screen agglutination negative. This organism was confirmed as being mec A negative by ESR.

MAMSA was used in conjunction with our conventional methods. From 189 specimens we had 15 MRSA isolates. Overall the results compared very well and suspect colonies were able to be picked off from both media at 24 hrs. While there were no significant differences in isolation rate the MAMSA was easier to read and is worth continued evaluation.

Strange Bedfellows

Rosemary Burnand - Diagnostic Medical Laboratory

A variety of uncommon organisms isolated in the first months of 2000 at Diagnostic Medical Laboratory.

Case 1: *Nocardia brasiliensis* isolated from a patient who cut his knee while gardening.

Case 2: *Haemophilus aphrophilus* isolated from a patient who developed a wound infection following a laminectomy.

Case 3: Three Samoan patients with infected tattoos - All three isolated *Staphylococcus aureus* (not MRSA). All three isolated *Streptococcus pyogenes*. Two of the three isolated *Arcanobacterium haemolyticum*. All three isolated non-toxin producing strains of *Corynebacterium diphtheriae* (two identified as *C. mitis*, the other identified as *C. gravis*).

Where our first two cases isolated significant pathogens, our third case was a mixture of bedfellows, the significance of some being in doubt.

Mantoux Testing and Monitoring of Staff for Tuberculosis

Vivienne Talbot - Health Waikato

Less than 10 years after the discovery of *Mycobacterium tuberculosis* was published by Koch (1882), he reported on his attempts to purify the components released from the culture media by *M. tuberculosis*. The product was later known as "Koch's old tuberculin", the beginnings of tuberculin and the mantoux test. Munday and Seibert, and later Seibert and Glenn, purified and standardised tuberculin to form PPD purified tuberculin, which is today used in the mantoux test.

The mantoux is a diagnostic test for latent tuberculosis infection (LTBI). Tuberculin injected intradermally forms a delayed hypersensitivity response and hence induration if the patient has been exposed to *M. tuberculosis*. The interpretation of induration and a positive test is based on cut-off values based on age, immunostatus, BCG vaccination, and country of origin.

For the mantoux test to be a reliable test, it needs to be performed with technical accuracy, as set out in the guidelines for Control of Tuberculosis in NZ, (Ministry of Health, 1996). In 1998, Health Waikato laboratory standardised the mantoux test according to the guidelines, and at the same time Health and Safety service put in place a new policy for monitoring staff for tuberculosis.

The policy for monitoring staff for tuberculosis covered three groups of employees:

- staff in monitored units (places of work which have an immediate risk ie >6 cases of tuberculosis)
- all other Health Waikato staff
- pre-employment staff

Monitored unit staff have an annual health assessment, and annual mantoux if not previously positive. A mantoux of 10mm or greater is considered positive and all staff are followed up with annual health questionnaires, chest x-rays, and referral to a respiratory physician if required. All staff are followed up for two years after leaving a monitored unit. General Health Waikato staff are given information on tuberculosis and there is an emphasis placed on education. All staff entering Health Waikato are given a baseline mantoux and are followed up as required.

In summary, Health Waikato have put in place a standardised mantoux method in accordance with the guidelines, and have implemented a staff screening policy for tuberculosis.

A Case of the Unexpected

Howard Verity - Medlab Timaru

A 59 year old man immunocompromised by diabetes and chronic renal failure and also suffering from heart disease was admitted to hospital with an extremely painful knee caused by gout. He died 41/2 days later of *Listeria monocytogenes* bacteraemia and meningitis.

The presentation of infection with this organism is often very variable and the subtlety of its presentation in this case was masked by the patient's other diseases, and possibly by the treatment he received for his inflamed knee. Information concerning origins of infection, predisposing conditions, incubation periods, treatment and epidemiology was also presented.

Abstracts from articles in the *Australian Journal of Medical Science*, the official publication of the Australian Institute of Medical Scientists.

Saleh FH, Jurjus AR. A comparison of the effects of two classes of anti-hypertensive drugs on the renal and cardiac systems of hypertensive rats. *Aust J Med Sci* 2000; 21 (3): 4-11.

Abstract: The aim of this study was to compare the effects of controlling angiotensin II (Ang II) and/or blood pressure (BP), directly at the inception of hypertension, on the cardiovascular and renal systems of spontaneously hypertensive rats (SHRs) using two different classes of antihypertensive drugs (enalapril and nifedipine). Our results showed that although both enalapril and nifedipine levelled BP to almost normal values, only enalapril was able to prevent the increase in weights of the kidneys and of the left ventricle of the heart ($P < 0.001$ wet and dry; $P < 0.04$ wet, $P < 0.001$ dry respectively) and the increase in the weight of the aortic arch and in the thickness of its media ($P < 0.001$ wet and dry; $P < 0.001$, respectively) seen in untreated SHRs. This was associated with a larger lumen diameter of the aortic arch ($P < 0.001$). Neither drugs had any effects on plasma renin activity. Our results support previous observations that BP is not the only factor behind some of the pathological changes in hypertension. Tissue Ang II level may play a major role as well.

Key words: hypertension, enalapril, nifedipine, left ventricle, kidney, aortic arch, rats, inbred SHR.

Melrose W, Smith J, Murray-Smith S, Zischke K, Covey J. Three cases of imported Bancroftian filariasis in North Queensland. *Aust J Med Sci* 2000; 21 (3): 12-3.

Abstract: An imported case of Bancroftian filariasis was diagnosed during malaria screening in a Papua New Guinean student attending

school in Charters Towers. Testing of a further 480 samples from PNG students in North Queensland revealed a further two cases.

Key words: filariasis, malaria, fever.

Kershaw G. An evaluation of one functional and two immunological assays for protein S in plasma. *Aust J Med Sci* 2000; 21 (3): 14-23.

Abstract: Protein S is an anticoagulant plasma protein whose measurement has proved problematical. Two antigenic methods and one functional method for measuring plasma protein S were evaluated in a large teaching hospital laboratory. Helena gels were used in the Laurell rocket procedure, and the Thrombonostika S used for the ELISA. In both cases free protein S was separated from bound protein S by PEG precipitation prior to the assay. Functional protein S was assayed by the APTT-based Staclot S kit on the STA Compact analyser. There was good overall correlation between methods ($r = 0.800$ for Laurell vs ELISA, $r = 0.782$ for Laurell vs functional, $r = 0.839$ for ELISA vs functional, $n = 95$). The antigenic assays were more labour intensive than the functional assay, particularly the Laurell method, but had the advantage of being unaffected by interfering substances. Of 10 samples with lupus anticoagulant activity 2 were classified as protein S deficient by ELISA but normal by the functional assay. In selecting a protein S method for routine use the advantages and limitations of each method should be considered, and each laboratory should establish its own reference range for the method chosen.

Key words: Protein S, ELISA, Laurell rocket, functional.

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The New Zealand Journal of Medical Laboratory Science, henceforth termed the Journal, is the official publication of the New Zealand Institute of Medical Laboratory Science (NZIMLS) and will consider for publication any paper relevant to the field of Medical Laboratory Science. This includes the disciplines of Transfusion Medicine, Clinical Biochemistry, Haematology, Microbiology, Histopathology, Immunology, Molecular Biology, as well as related areas of interest to medical laboratory scientists, including epidemiology, public and community health, management, toxicology, pharmacology, education and so on.

Papers submitted to the Journal are in the form of:

- Original articles
- Review articles
- Brief Communications
- Technical notes
- Letters to the Editor
- Research letters

Articles submitted for publication are understood to be offered only to the Journal and must have not been previously published except in the form of an abstract. Accepted articles become the property of the Journal with copyright vested in the NZIMLS.

Summary of requirements and preparation of manuscripts

All articles must be submitted in hard copy form (as described below), and if possible on a computer disc in the form of a word processor file (please state type used), or as a file attachment by e-mail to the Editor (address below).

Submit two copies of the manuscript on A4 white bond paper. margins should be at least 2.5 cm. Authors are advised to retain a copy of all material submitted. Type manuscripts double-spaced throughout on one side of the paper only. Number pages consecutively commencing with the title page.

Each manuscript component must begin on a new page in the following sequence:

- Title page
- Abstract and key words
- Introduction
- Materials and methods
- Results
- Discussion
- Conclusions
- Acknowledgments
- References
- Tables
- Illustrations

Submit the complete manuscript in duplicate (plus computer disk if possible) to the Editor: Rob Siebers, Department of Medicine, Wellington School of Medicine, PO Box 7343, Wellington South, Wellington (e-mail: rob@wnmeds.ac.nz) together with a covering letter signed by all authors stating that the submitted article is original, is not

under consideration for publication elsewhere nor has it previously been published, that references cited in the article have been checked against the original or appropriate data bases (such as Medline), and that in the case of multi-authorship all authors have contributed directly to the planning, execution of the study, analysis, or to the critical writing of the article. All authors must approve the final version of the article.

Articles submitted to the Journal are peer-reviewed, acceptance is at the discretion of the Editor. No undertaking is given that any article will be published in a particular issue of the Journal. Contributors are responsible for the scientific content and views. The opinions expressed in the Journal are not necessarily those of the Editor or Council of the NZIMLS.

Preparation of manuscripts

Title page

The title page should contain a concise title of the article not exceeding three lines, including punctuation and spacing. All authors must be identified with first name, middle initial and last name of each author, with highest academic degree(s) and position held. Include the name of the institution with which each author is affiliated. Supply a complete address (including e-mail) for the corresponding author and indicate who this is.

Abstract and key words

Abstracts should be about 250 words, contain concise and precise information, and be structured under the following sub-headings: **Objective(s), Method(s), Result(s), Conclusion(s)**. Three to ten key words are to be listed below the abstract. Authors are advised to comply with approved terms from the Medical Subjects Headings list from Index Medicus.

Text

The style of writing should conform to acceptable English usage. Do not use slang, medical jargon or unnecessary abbreviations. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement. Report measurements in the units in which the measurements were made, preferably the International System of Units (SI).

Where possible, observational or experimental articles should be divided into sections headed:

- Introduction
- Materials and Methods
- Results
- Discussion
- Conclusions
- References

Long articles may need subheadings within some sections, especially the methods, results and discussion sections to clarify their content.

Introduction

Clearly state the purpose of the article. Summarise the rationale for the study or observation. Give only strictly pertinent references, and do not review the subject extensively.

Materials and methods

Describe the selection of the observational or experimental subjects (patients or experimental animals, including controls) clearly. Identify the methods, apparatus and procedures in sufficient detail to allow

experienced scientists to reproduce the results. Give references to established methods. Adequately describe new or substantially modified methods. Describe statistical techniques employed to analyse the results, include references where appropriate. State if ethical approval has been obtained where appropriate. Identify precisely all drugs and chemicals used, including generic names, dosages and routes of administration. Do not identify patients or hospitals or institutions without consent.

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Present results in a logical sequence in the text, tables and illustrations. Do not repeat in the text all the data in the tables or illustrations. Emphasise or summarise only important observations (both positive and negative).

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Indicate the new and important aspects of the study and emphasise the conclusions that follow. Do not repeat in detail data given in the Results section. Include in the Discussion section the implications of the findings and their limitations and compare the observations to other relevant studies. Link the conclusions with the aims of the study. Avoid unqualified statements and conclusions not fully supported by the data. Avoid claiming priority and alluding to work that has not been completed. State new hypotheses when warranted, but clearly label them as such. Recommendations and suggestions of further studies may be included if appropriate. State limitations of the study, if any.

Acknowledgments

Acknowledge the people who have made substantive contributions to the study. Acknowledge organisations or commercial firms who have made finance, equipment or supplies available for the study. Authors are responsible for obtaining consent from everyone acknowledged by name as readers may infer their endorsement of the data and conclusions.

References

Throughout the body of the manuscript number references consecutively in the order in which they are first mentioned and identify references in text, tables and legends by Arabic numerals in parentheses, e.g. (1), (2,3) or (4-6).

When citing authors in the text, where there are three or more authors, acknowledge only the first author, e.g. Smith et al (1999). Where there are only two authors acknowledge both, e.g. Smith and Brown (1999).

Use the Vancouver style for citing references. The format for this is to list all authors by last name followed by their initials (no more than two). If there are six authors or less, list all, if more than six, list the first six followed by "et al". Next state the full title of the cited reference, followed by the Index Medicus approved journal abbreviation (in italics), the full year of publication, semicolon, volume number (include supplements in parentheses), colon, and first and last page numbers. For example:

1. Sawyer G, Kemp T, Shaw R, Patchett K, Siebers R, Lewis S, et al. Biological pollution in infant bedding in New Zealand: high allergen exposure during a vulnerable period. *J Allergy Clin Immunol* 1998; 102: 765-70.

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Type each table double-spaced on a separate page. Do not submit tables as photographs. Number tables consecutively and supply a brief title for each (at top of the table). Give each column a short or abbreviated heading. Place explanatory matters in footnotes, not in headings. Explain in footnotes all non standard abbreviations used in each table. Do not use vertical lines in tables.

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Submit two complete sets of figures. Figures should be professionally rendered and photographed, or generated by a good graphics program on a laser printer; free-hand or typewritten lettering is unacceptable. Instead of original drawings, X-ray transparencies or other material, send sharp, glossy black-and-white photographic prints, usually 12.7 by 17.3 cm, but no larger than 20.3 by 25.4 cm. Illustrations will be accepted for reproduction in colour if they are deemed essential (e.g. haematology, immunology, histopathology stains), obtain prior permission from the Editor.

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Abstracts of Presentations at the NZIMLS ASM, Rotorua, August 2000

The Social Changes/Aging Populations

Craig Lehmann, PhD, Dean, School of Health Technology & Management, Health Sciences Centre, State University of New York, USA

The presentation will discuss the aging population in North America and the economics of providing health care. The presentation will focus on population trends, case mix and the relevance of early detection of disease. Data demonstrating a shift from hospital inpatient to ambulatory care will be presented along the growing utilisation of home health care. The results from a study evaluating utilisation of diagnostic services of one of the largest home health care providers in New York State will also be presented.

The Need for Change in the Funding of Community Referred Laboratory Testing

Philip Pigou, Project Manager, Health Funding Authority, Wellington

The Health Funding Authority and previously the Regional Health Authorities and the Transitional Health Authority have consulted, at various stages through the mid to late 1990s, on strategies developed to manage the funding of community referred laboratory testing. Little significant change has been implemented in this sector as a result of these consultation processes.

There has been a range of reasons put forward for consulting on these strategies. These have included:

- Arguments that there has been a lack of evidence that the prices paid by the HFA reflect the cost of an efficient and quality laboratory service in New Zealand;
- Arguments that there has been a lack of competition in the sector in relation to the price of laboratory testing;
- The creation of regional discrepancies in contractual terms and conditions, including what tests are available on the community referred schedule;
- Significant increasing levels of expenditure on laboratory testing.

These consultation and analytical processes have resulted in the development and implementation of a range of initiatives. These include:

- The implementation of quality standards in laboratory contracts.
- The development of a national laboratory test schedule to replace the four different schedules used by the former regional health authorities (RHAs). This will allow people throughout the country to have the same level of access to the same free laboratory tests. With the new schedule, the HFA will purchase the most appropriate range of tests, in the most appropriate way.
- Implementation of the national laboratory test schedule is expected to include access to the schedule by Hospital and Health Services (HHS) laboratories. Allowing HHS laboratories to access the schedule is one step to removing some artificial barriers between these areas and improves access to services. Other proposals to remove barriers and facilitate fair competition are being negotiated with HHS and community laboratories.
- Examination of how much to pay for each laboratory test on the schedule. Around \$178 million was spent on community-referred laboratory testing in the 1998/99 financial year, representing about 25 million tests.

A more in-depth discussion of the reasons for change and the implementation of change will be presented.

Immuno-Biology of Cancer: An Overview from the Histological, Haematological and Genetics Perspectives

Dr H Pullon, Dr N Scobie, Dr M Dray, Barbara Harrison, Heather Goddard, Health Waikato, Hamilton

Current approaches in clinical diagnosis, aimed at giving clinicians the diagnostic information they require, is best served by a multi disciplinary approach.

This involves the planning of good biopsy material, correctly handled and subsequently processed in the relevant disciplines.

Each discipline will describe their specimen requirements and preparatory procedures. Routine histology, cytology and immuno-histochemistry, bone marrow and flow cytometry, cytogenetics, and occasionally molecular analysis are undertaken.

The principles of analysis employed will be discussed and collectively demonstrated by relevant case reviews.

Optimal cancer treatment requires a compilation of the results supplied from all pathology disciplines, which culminates in the correlation of treatment outcomes based upon accurate diagnoses.

Primary Amoebic Meningo-Encephalitis caused by *Naegleria fowleri*

Matthew Akehurst, Microbiology Department, Health Waikato, Hamilton

Naegleria fowleri is the main protozoan causing primary amoebic meningoencephalitis in humans. In New Zealand primary amoebic meningoencephalitis has mainly been implicated with swimming in hot pools. It enters the brain via the Cribriform plate through water entering the nasal cavity. Incubation period is usually 2-3 days; there is an abrupt onset of symptoms including fever, stiffness of the neck, fixation of the pupil and nausea thus mimicking bacterial meningitis. Differential diagnosis from bacterial meningitis can be achieved by heating a wet film and observing the characteristic movement of the amoebae. Amoebae can be mistaken for leukocytes in a counting chamber, therefore most cases of primary amoebic meningoencephalitis are diagnosed on post-mortem. It is recommended to treat with Amphotericin B, however the outcome of the disease is very poor with only about a 1% survival rate.

Development of Anti-filarial antibodies in a Group of Expatriate Mine-Site Workers with Varying Exposure to the Disease

Wayne Melrose, School of Public Health & Tropical Medicine, James Cook University, Townsville, Australia

Thousands of American servicemen became infected with Filariasis during the Second World War and a number of cases of filariasis among missionaries, expatriate workers and travellers have been reported in the literature. Despite this, lymphatic filariasis is seldom thought of as a threat to expatriates working in, or travellers to filarial-endemic areas. This study investigated the development of IgG1 and IgG4 anti-filarial antibodies in a group of expatriate mine-site workers working for varying lengths of time in a filarial-endemic area. IgG1 and IgG4 filarial antibodies and filarial antigen were measured in a group of expatriates who had been working at a mine-site in a filarial-endemic region of Papua New Guinea for between 1 and 8 years. Fifty nine percent of the workers developed IgG1 antibodies and the level of antibody correlated with the length of exposure. Six subjects developed IgG4 antibody suggesting active filarial infection. None of the subjects showed filarial antigenaemia. This study quite clearly shows that filariasis is a threat to expatriate workers and travellers especially if their work environment and lifestyle constantly exposes them to mosquito bites and should be considered when investigating illness in returnees from filarial-endemic

ic areas. As there is no proven prophylaxis against filariasis, the only effective means of control is protection from mosquito bites and a control program in the local population to minimise expatriate exposure.

Brachylaima, a New Human Trematode Infection in Australia

Andrew Butcher, Institute of Medical and Veterinary Science, The Queen Elizabeth Hospital, Dept of Microbiology and Infectious Diseases, Woodville, Australia

Brachylaima spp. (Digenea: Brachylaimidae) are intestinal trematode worms of mammals, birds, reptiles and amphibians with land snails acting as the first and second intermediate hosts. There had been no reported human infections until those described by Butcher et al. in two children in 1996 and an adult in 1998. There has been a further 3 cases all having contracted the infection by eating infected helioid land snails. The introduced European helioid land snail or white snails as they are commonly known, are introduced pests in many parts of Southern and Western Australia. These snails have reached alarmingly high numbers in many parts of South Australia, which has resulted in greater human contact. As the majority of snails harbour the infective larval stage of the *Brachylaima* fluke worm accidental ingestion can result in an infection. This parasite is an example of a new human disease and the clinical features and diagnosis of each infection will be presented along with the description of the experimental life cycle.

Pigs, Pole Toilets & Pek-Pek - A parasitologist in Paradise

Wayne Melrose, School of Public Health and Tropical Medicine, James Cook University, Townsville, Australia

Another light hearted look at field work in the land of the unexpected - Papua New Guinea where taking blood off ex (hopefully!) Head hunters in the dark of the night, falling into and out of toilets and being chased by pigs is all part of the job. There is a serious side though - the presentation will highlight the danger that porcine-associated zoonotic diseases and poor sanitation pose to Papua New Guinea.

Modern Microbiology and Trends in Infectious Diseases in New Zealanders

Associate Professor Rod Ellis-Pegler, Clinical Director, Infectious Disease Unit, Auckland Hospital

There are global concerns about the spread of old infectious diseases, the development or recognition of new ones and the emergence and spread of antimicrobial resistance. While New Zealand has been spared some particular examples of these infectious diseases e.g. Lyme disease, ehrlichiosis and West Nile fever, the same trends are nevertheless evident here.

Tuberculosis is increasing here again. Modern fingerprinting methods allow precise recognition and a better understanding of local mini epidemics. A type B meningococcal epidemic has been running for a decade: again, modern methods allow us to specify the principal epidemic strain with precision. The numbers of those infected with HIV increase every year here. Modern methods document the viral changes within an infected individual, even challenging our concepts of what organism identity is. We see resistance of HIV to all classes of anti-retrovirals, again demonstrated by modern and unfortunately very expensive methods.

Improving technology leads to increasing knowledge, which leads to better decisions.

Medical Science Re-discovers the Parasite

Wayne Melrose, School of Public Health & Tropical Medicine, James Cook University, Townsville, Australia

For a long time parasitology has been the "poor relation" of the communicable disease family overshadowed by the discovery of new bacteria and viruses, the increasing problems of bacterial antibiotic resistance and global epidemics of organisms such as HIV. In recent years, there has been a rapid upsurge in interest in human parasitology, fuelled by several factors such as the discovery that parasites such as *Trichuris* and *Ascaris*, even when present in low numbers, can have adverse effects on children's growth and cognitive ability, and that "asymptomatic" filarial infection causes renal disease and immunosuppression. The introduction of single annual dose mass treatment to control filariasis and intestinal helminths, means that global eradication of these parasites is possible within the next 25 years or so. The low cost and the need for only a limited infrastructure makes the sponsorship of these programs attractive to mining companies and other private sector commercial entities who are giving increasing amounts of funding to parasite research and control. The upsurge in travel to exotic locations especially "ecotourism" ventures where participants get close to nature, and the influx of people migrating from, or being displaced from parasite endemic countries has caused a dramatic increase in the number of parasite infections seen in countries such as Australia and New Zealand.

Viral Meningitis Outbreak Due to Echovirus Type 33

D Hulston, J Shewan, S Huang, ESR, Porirua; J Lindeman, Waikato Hospital; J Cull, MC Croxson, Auckland Hospital

New Zealand is currently experiencing an aseptic meningitis outbreak caused by Echovirus type 33 (E33). E33 (family, Picornaviridae) is recognised as a causal pathogen for aseptic meningitis, respiratory disease, gastroenteritis, rashes, undifferentiated fever and abortion in pregnant woman. Enteroviruses are acquired by faecal-oral transmission. The aseptic meningitis outbreak appears to have started in the Waikato at the end of March 2000, then spread to Auckland and Northland. So far we have received nearly 60 isolates for typing from these regions. We have confirmed that 48 isolates were E33 (Waikato (34), Auckland (13), and Northland (at least 1).

Viruses were isolated in tissue culture from CSF, throat swabs, faeces and urine in Auckland and Waikato Virus laboratories and forwarded to ESR for further typing. The Auckland and ESR virology laboratory did initial typing by neutralisation tests with LBM pool, which suggested probable E33. However, the ESR confirmatory neutralisation tests by RIVM pool, Cox A pool and various monospecific antisera did not give conclusive results. Twenty-six isolates were sent to CDC-Atlanta for molecular typing. CDC sequenced around 360 nucleotides in the 5' portion of the VP1 gene. The results showed that the percent of nucleotides of NZ isolates was 78 % identical to the prototype E33 strain. This suggests that the virus has drifted and the current antisera (raised against the prototype viruses 30-50 years ago) used in the neutralisation tests could not sufficiently neutralise the virus. Some isolates were also sent to WHO regional reference laboratory in Melbourne and the molecular typing showed they are E33.

Critical Incident Stress Debriefing (The Lakeland Health Model)

Ray Bloomfield, Co-ordinator CISM, Lakeland Health, Rotorua

This session will look at the role that debriefing plays in Critical Incident Stress Management - Peer Support, Defusing, Debriefing. Definition of a Critical Incident: "Any event which has sufficient emotional power to overwhelm a person's usual ability to cope." Jeff Mitchell Ph.D The session will look at incidents that may suggest the need for Debriefing, as well as the goals of Debriefing. A very important goal is to accelerate the normal recovery of normal people who are suffering through normal but painful reactions to abnormal events. Even though the feelings and emotions that are being experienced are painful, they are normal, and that knowledge helps in the recovery process.

Some of the common signs and symptoms of distress will be identi-

fied. Rules for running a debriefing session will be discussed, and the session will identify and go through the 7 steps of the Mitchell Model that is used at Lakeland Health Ltd. Debriefings occur between 24 - 72 hours after a critical incident. Often within the first 24 hours, people are in shock and benefit more from Peer Support and Defusing - a less formal process than debriefing. The session will also look at the follow up support and care of staff following a debriefing.

Occupational Safety and Health Management

Dr Max Robertson, International Accreditation New Zealand, Auckland

The Health and Safety in Employment Act 1992 places the onus on employers to take all practicable steps to prevent injury or harm to their staff and to be able to demonstrate that they are doing this.

Laboratories need some formal management procedures to ensure that they have identified all workplace hazards and are managing them in such a way as to minimise the risk of harm to staff.

This paper will identify the key requirements of the Act and set out the elements of a health and safety management system that ensures the systematic management of work place hazards. It is important to integrate health and safety management into the ISO/IEC Guide 25 quality management system of your laboratory.

Not only can you reduce the possibility of the Chief Executive going to gaol or of paying large rehabilitation costs, you will have happier and healthier employees who will work more effectively. And you may even pay considerably less ACC levies if your health and safety management system has been assessed.

Cord Blood Banking - Recycling Stem Cells for Transplantation

Dr Robyn Rodwell, Haematology Division & Queensland Cord Blood Bank, Mater Misericordiae Hospitals, South Brisbane, Australia

Haemopoietic stem cell transplantation (SCT) using bone marrow (BM) the traditional source of graft, offers potentially curative therapy for an expanding range of haematologic, malignant, hereditary and immunological disorders. Despite the development of large volunteer bone marrow donor registries, approximately only half of the candidates eligible for this therapy have a suitable HLA matched related or unrelated donor. Animal and laboratory studies performed in the 1980s showed that umbilical cord blood (UCB) was a rich source of stem cells that could substitute for bone marrow in SCT. In 1988 and 1993 respectively, the first successful "related" and "unrelated" UCB transplants were performed. Cord blood banking is a novel enterprise arising from these studies, whereby stem cells from UCB collected after childbirth can be recycled and cryopreserved as a readily available frozen source of HLA-matched donors. An international network of cord blood banks has evolved to meet the demand for stem cell donors. Data from a recent landmark study show that UCB provides an equally effective source of stem cells as BM for use in transplantation. Due to the finite number of cells available for transplantation, most data to date has been derived from studies in children. Cord blood has several advantages over bone marrow as a source of graft. It can be easily procured without risk to the mother or baby; a greater degree of mismatch between donor and recipient is tolerated; and there is a reduced incidence of life-threatening complications such as graft versus host disease and infections post-transplantation. This is because the donor is a newborn infant with an immature system and a low risk of latent viral infection. A cord blood bank with a frozen reserve of HLA typed UCB units provides a readily available source of product and reduces the lag time between arranging and performing a transplantation. The major disadvantage of UCB with respect to bone marrow is the limited number of cells due to the finite volume available. The process of cord blood banking involves six basic steps: (1) providing information and obtaining donor consent; (2) collection of the UCB; (3) collection of maternal blood and a medical history; (4) processing including volume reduction and freezing of the UCB units; (5) labora-

tory testing of cord and maternal blood samples and; (6) data storage and donor searches based on HLA typing. Ethical, legal and regulatory issues need to be considered in the design of a cord blood-banking programme. Ex vivo expansion of UCB stem cells will be necessary before this therapy can be universally applied in adults.

Myelodysplastic Syndromes - Peripheral Blood and Bone Marrow Morphology

Dr Elayne Knottenbelt, Haematologist, Medlab Central, Palmerston North

The morphological diagnosis and classifications of the Myelodysplastic Syndromes (MDS) continue to be modified and extended to include the many cases which do not easily fit the criteria of the original FAB classification introduced in 1982. Specific cases will be used to demonstrate the criteria now used with particular emphasis on the overlap with the Myeloproliferative disorders and the recent modifications suggested for diagnosing Chronic Myelomonocytic Leukaemia and the Refractory Anaemias.

The importance of not using the Myelodysplastic Syndromes as a wastebasket for undiagnosable haematological abnormalities will be emphasised by including the differential diagnosis of MDS.

Apoptosis - Mechanisms and Relevance to Haematological Disorders

Robin Allen, Chief Scientist Haematology, Waikato Hospital, Hamilton

There are two modes of cell death, namely apoptosis (programmed cell death) and necrosis (accidental cell death). Apoptosis is an active mode of cell death controlled by complex interactive processes within the regulatory mechanisms of the cell.

Apoptosis is an integral part of life and is involved in many physiological processes. Aberrant apoptosis plays a significant role in the pathogenesis of many diseases. There are diseases with reduced apoptosis, such as cancer or autoimmunity, and diseases associated with excessive apoptosis, such as AIDS. The failure of apoptosis as an underlying cause of neoplasia has particular relevance to haematological malignancies. Mutations and deletions of apoptotic genes play important roles in carcinogenesis, tumour growth and tumour regression. Genes that have received the most attention are Bcl-2 and p53. In addition, modulation of apoptosis is exploited in some chemotherapeutic approaches.

As the majority of the events that characterise apoptotic cell death can be demonstrated by multiparameter flow cytometry, a significant number of methods have been described to analyse and quantify the apoptotic process by this type of analysis.

Managing Diagnostic Services in a Decentralised Community-Based System

Craig Lehmann, PhD, Dean, School of Health Technology & Management, Health Sciences Centre, State of University of New York, USA

The presentation is intended to provide individuals with an overall understanding of why there is an emphasis to decentralise health care to a community-based system. A detailed overview of sub-acute nursing homes, assisted living, and home health care will be presented as well as the role of laboratory diagnostic in a community-based system. The session will then focus on the economics of providing diagnostic services in this arena, which will include integrated delivery systems. The final portion of the programme will discuss the role of informatics and will provide a detailed presentation of a new informatics system designed to the needs of a community-based system as well as laboratory diagnostic services.

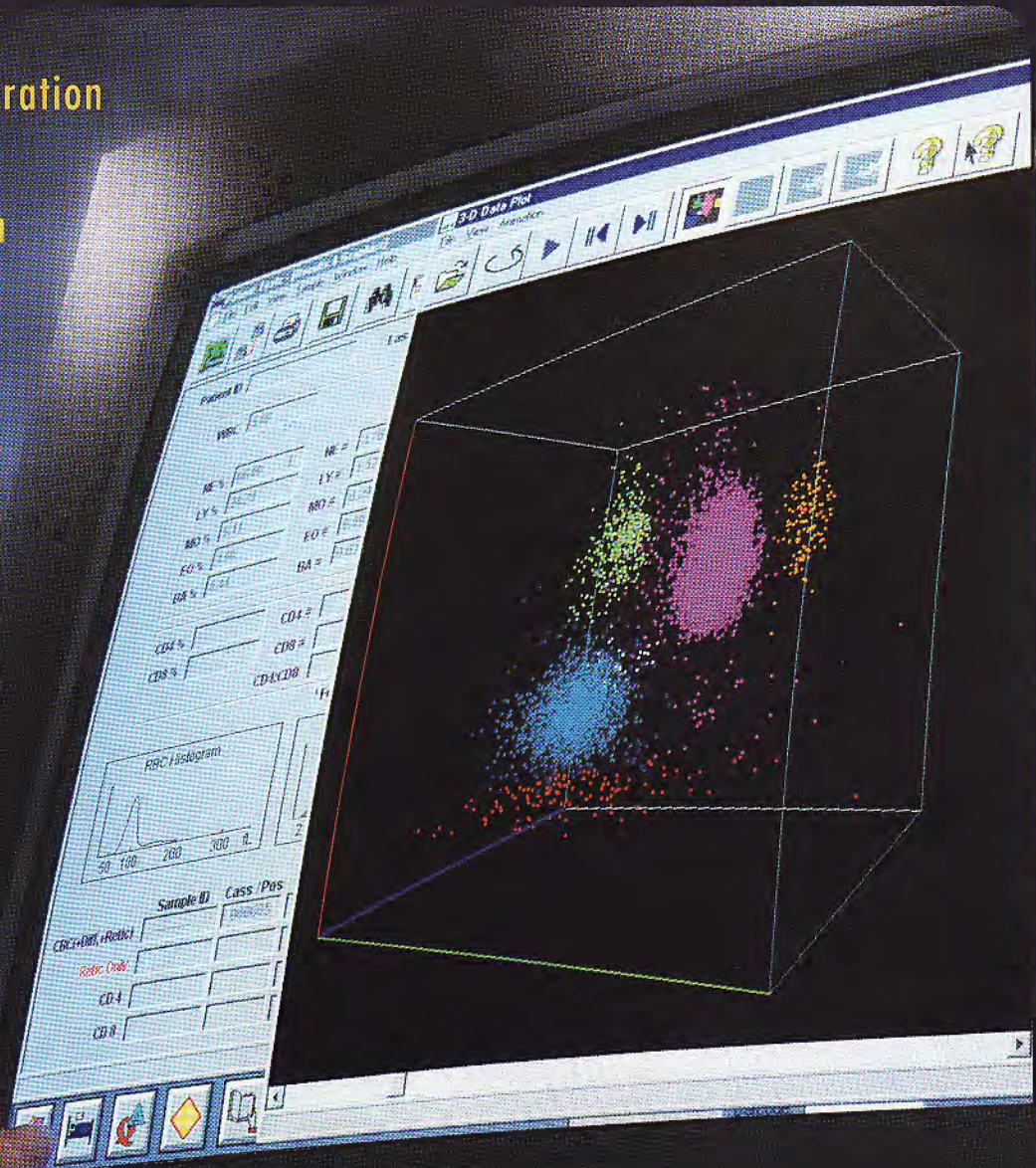
Expected outcomes: At the conclusion of this session, participants will be able to describe the changing elements of health care and why

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there is an emphasis to decentralise to a community-based system; 2) list the economic benefits of providing diagnostic services in a variety of settings (ie. home health care); and 3) list the impact of point of care testing, image technology and informatics will have on the delivery of diagnostic services in a community-based system.

The Creation of a Core Laboratory: The Bay of Plenty Experience

Linda Smith, Laboratory Manager, Hospital Site, Medlab Bay of Plenty

Medlab Bay of Plenty is a community laboratory providing diagnostic services for the Bay of Plenty. In July 1996 the laboratory underwent a metamorphosis, from a traditionally organised departmental laboratory into a Core Laboratory.

This change was precipitated, when a five-year contract to provide services for Tauranga Hospital was realised. The two laboratories were integrated. The physical transition was performed overnight.

The small size of the existing laboratory at Tauranga Hospital forced the new laboratory organisation into two physical operations. Haematology, Biochemistry, Immunology, Blood Transfusion and a small Stat Microbiology were merged into an open plan Core Laboratory at the Hospital site. Central Specimen Reception was also moved to this site. Microbiology, Histology and Administration remain at our 1st Ave premises.

Moving analysers closer together and knocking down physical walls is not difficult to achieve. Platform consolidation - the buzzword for Core Labs - is vital for the efficiencies that are gained by the formation of a Core Lab.

However this overview is not about the physical aspects of Core Laboratories. The managerial and workplace advantages and disadvantages of this new laboratory structure will be discussed.

1999 - A Year of Considerable Change for Diagnostic Laboratory and Medlab

Dennis Reilly, Manager Biochemistry Immunology Department, Diagnostic Medlab, Auckland

On the 27th May Diagnostic Laboratory and Medlab signed an agreement to merge their operations as from the 1st July 1999. A new company DML was formed which pools the resources in the newly created DML. The Diagnostic Directors had a 70% shareholding with SGS, a 30%, which was based on market share.

Later in the year, 1st December 1999, DML was merged into the newly expanded Sonic Healthcare group. Sonic Healthcare has over 7,000 people in the group spread widely through Australia and New Zealand.

This paper will outline the steps involved in the process of bringing together two large community laboratories.

Laboratory Integration; a Model for Small Isolated Laboratories

Trevor Rollinson, Southern Community Laboratories, Dunedin

The successful integration of small isolated laboratories into a large regional laboratory is dependent upon a number of systems already in use being extended to provide the services the laboratory requires. These include the information system made up of the computer to process results, communication systems and the laboratory quality system.

The most critical component to the successful integration is the availability of staff their training and acceptance of the change. Commonality of equipment, procedures and documentation minimises the training time of staff and gives the flexibility necessary in order to provide cover for annual leave and sickness.

A high level of support from the regional laboratory is necessary in the planning, set-up and initial start-up of the laboratory. Continued support at all levels from Management, Quality Management, Technical Heads and staff from the regional laboratory is necessary to

ensure the staff feel part of a larger team and the small laboratory does not become isolated.

A Social Audit of BMLS Clinical Experience

Holly Perry, Henriques, Paul, Auckland University of Technology, Auckland

Social auditing is the process whereby an organisation can account for its social performance, report on and improve that performance. It assesses the social impact and ethical behaviour of an organisation in relation to its aims and those of its stakeholders (1).

One of AUT's social objectives is to prepare students for their vocation. The fourth year of the BMLS degree aims to do this by consolidating theoretical knowledge with laboratory practice in the clinical placement.

A social audit was conducted to assess how well the social objectives are met. The audit took the form of a structured interview with the three stakeholder groups; students, laboratory staff involved in training and AUT BMLS lecturers.

20 students were interviewed by telephone in the student group, and comprised a mixture of currently placed students and recent BMLS graduates. 10 laboratories currently involved in clinical placement training were interviewed in the laboratory group. The AUT lecturers were interviewed together in a staff meeting. Questions and results of the audit will be presented.

This is your opportunity to hear what students do and do not like about the clinical experience in your labs, and to make suggestions as to how AUT can better meet your social objectives and expectations.

(1) Pearce, J. *Community Enterprise Consultancy and Research, UK*

Re-engineering the Clinical Laboratory for the Millennium

Craig Lehmann, PhD, Dean, School of Health Technology & Management, Health Sciences Centre, State University of New York, USA

This session will discuss how to re-engineer the clinical laboratory for the next Millennium. Creation of departments such as "Chematology" will be a necessary component as well as "Connectivity" throughout the integrated delivery system, State, Nation and/or Country. Utilisation of image technology and the standardisation of laboratory information systems will play a vital role in the delivery of diagnostic services. A detailed discussion will be presented on the use of image technology in haematology as well as examples of economic benefits of information connectivity.

Automation.....Juggernaut or Opportunity

Helen Martin, Deputy Head, Clinical Biochemistry, Gribbles Pathology, South Australia

Labour is the largest cost centre for any pathology laboratory, constituting over 50% or even up to 70% of the budget and until the nineties cost containment was largely synonymous with reduction in staff numbers. Since that time more mature appraisal has revealed many other avenues for saving the ever diminishing health dollar but automation remains the major enabler in the quest for cost containment.

Laboratory "automation" in the late 1950's meant introducing stand-alone instruments to automate previously manual analytical processes. Technology continued to advance and by the early 1980's, the first totally automated laboratory was implemented in Japan. The concept of a core laboratory was developed as a means of creating a more efficient and cost effective environment for urgent test delivery. Lately, attention has been focussed on the "front end" of the laboratory. Clearly automation both as a concept and in its specific guises has evolved significantly, however, many challenges remain.



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Laboratory managers and regulatory bodies need to re-evaluate appropriate routine practice in the context of the advances made by automation, laboratory information systems need to be optimised, professional groups need to re-evaluate their relevance and our industry colleagues are also facing new challenges.

Scientists should now be re-evaluating the ways in which they can contribute to the modern laboratory. Far from requiring an accordion of mindless button pushers, I believe that a cost effective laboratory needs staff who can not only push buttons in the correct sequence but who can contribute to value added activities such as method assessment, technical trouble shooting, client liaison and test interpretation. Automation should not be feared as a juggernaut but should be embraced for the opportunities it provides.

The Health Reforms: Hospital Outcomes

Dr Ross Boswell, Middlemore Hospital, Auckland

The New Zealand Health Reforms triggered by Simon Upton's 1991 green-and-white paper have led to a more even redistribution of funding across population sectors, and towards less institutional delivery of health care. This has been achieved in part by greater emphasis on tracking the costs of care, but that has in itself increased the proportion of vote: Health expended on administration, and decreased the proportion expended on care delivery.

Prior to 1993, the costs of laboratory tests were only approximately known. In many hospitals their prices are now absolutely defined, but we may have lost sight of their values. This process has exposed flaws in the pricing models that have driven perverse behaviours. It has also made the introduction of new tests and procedures more difficult, since there has been little opportunity to float them when the demand has not been assured.

After 7 years, the situation seems to be stabilising. We have been through a "Rambo" phase of aggressive competition between health providers, and may be emerging to a new era of self-enlightened cooperation. It must be time for another round of reforms.

Plasma Fractionation at CSL Bioplasma

Dr Jerry Kanellos, Research and Development Manager, Haemostasis, CSL Bioplasma, Australia

The use of the Cohn fractionation process to achieve the separation of various protein fractions from whole plasma has been in use throughout the world for over 50 years. The process has proved reliable and has delivered these valuable therapeutic products to patients in need. Advancements in technology and the ever-increasing need to further enhance the safety and purity of therapeutic products have however demanded the adoption of new methodologies for the manufacture of these products. CSL embraced this change with the construction of a new plasma fractionation facility in Broadmeadows, Australia, a plant that has been designed to utilise the resolving power of chromatography on a large scale. This presentation will outline the benefits achieved to date using this approach and the prospects for the isolation of further plasma proteins of therapeutic value in the future.

The Use of Fractionated Products and Their Future

Dr Susanta Ghosh, Transfusion Medicine Officer, New Zealand Blood Services, Waikato, Hamilton

First fractionated product albumin, was introduced during the second World War. Since then we have seen the introduction of many other plasma proteins in regular clinical use. These products have saved many lives over the last half a century but at the same time we have seen the spread of HIV and HCV with these products, particularly among patients with clotting disorders. Development of viral-inactivated blood products has significantly decreased the risk but it is not zero-risk. While concerns arose with HIV and HCV, discovery of nvCJD has

emphasised the ever-existing risk with the use of plasma derived products and signals that in future, most protein products will be produced using molecular technique.

Clinical Trials of New Blood Product Technologies

Geoff Herd, Charge Scientist, Transfusion Medicine, Whangarei Hospital

This paper reviews some of the clinical trials of new blood products in the management and control of bleeding and sepsis. Recombinant thrombopoietin has been used to stimulate platelet production in patients on chemotherapy. Conjugated oestrogen compounds are now being used to control bleeding in a variety of disorders. Human antithrombin III has recently been evaluated in large (2300 patients) multicentre trial in the management of sepsis in intensive care patients. Mannose binding lectin (MBL) fractionated from human plasma has been used for the treatment of recurrent infection. A brief review of the role of MBL in infectious disease and its potential as a screening marker and as a replacement therapy for deficient individuals is examined.

The Applications of Psoralen S-59 within Transfusion Science

Darryn Knight, New Zealand Blood Service, Auckland

A photochemical treatment process has been developed for the inactivation of high titres of viruses and bacteria in blood components. It involves a psoralen, S-59, which once activated, binds with pyrimidine bases preventing DNA replication.

The Significance and Management of T cryptantigen Exposure in Hospitalised Populations

Dr Robyn Rodwell 1, Kerwick AM 1, Joyce A 2, S Pillai 2, Taylor K 1

1. Haematology Division, Mater Hospitals, South Brisbane, Queensland, Australia

2. Blood Bank Division, Mater Hospitals, South Brisbane, Queensland, Australia

T cryptantigen exposure (TCE) is a serologic complication of bacterial infections with diagnostic, prognostic and therapeutic implications. It can be recognised by a simple agglutination test with the peanut lectin *Arachis hypogea*. TCE occurs when bacterial enzymes expose the normally hidden T cryptantigens on the red blood cell (RBC) surface rendering them polyagglutinable by naturally occurring IgM anti-T antibodies. TCE occurs in approximately 0.5% of unselected and 6-7% of selected hospitalised patients. These include those with malignancies, acquired immunodeficiency syndrome (AIDS), surgical patients with bowel disorders or intra-abdominal infections, paediatric patients with *Streptococcus pneumoniae* infections in whom there is a high risk of a virulent form of haemolytic uraemic syndrome and in neonates with necrotising enterocolitis (NEC). In the latter group, the frequency of TCE ranges from 11-33%, it signifies fulminant disease, a high likelihood of Clostridial infection and surgical intervention, and mandates the avoidance of standard plasma containing blood products. If plasma infusion is unavoidable washed RBC and platelets and low titre anti-T (LTA-T) containing plasma products is recommended. We have successfully used high haematocrit RBC and LTA-T fresh frozen plasma and platelet products (kindly provided by the Australian Red Cross Blood Transfusion Service Queensland) in neonates with NEC. Our experience with two babies with evolving NEC with an initially negative test for TCE and who received standard blood products (one prior to transfer to our institution) confirm that inappropriate transfusion therapy may lead to severe intravascular haemolysis, disseminated intravascular coagulation and death.

The Supply of Blood to the NZ Army in East Timor

Christine van Tilburg, New Zealand Blood Services, Auckland

The New Zealand Blood Service supplies the New Zealand Defence force with blood support for the peacekeeping force in East Timor. A brief overview will be given of the procedure, protocols and problems involved in this on-going exercise.

Leucodepletion Trial in New Zealand Blood Service Waikato

Amanda Haywood, New Zealand Blood Service Waikato, Hamilton

A report on the trial conducted at NZBS - Waikato in May. The pilot trial enabled us to identify and document practical challenges that will be encountered when introducing leucodepletion into routine blood processing.

Percent Hepatitis A Positivity in the Donor Population

Julie Clark, New Zealand Blood Service Waikato, Hamilton

To share data collected when testing random donors for Hepatitis A antibodies at the Waikato Blood Donor Centre.

Clinical Use of Immunology Results

Dr John Petrie, Rheumatology & Physical Medicine, Lakes Care Medical Centre, Rotorua

Immunological tests play a vital role in the differential diagnosis of a variety of uncommon connective tissue diseases. Whilst they have little value in the monitoring of treatment, results do influence treatment choice and patient education.

The wide availability of such tests requires that clinical indications for their use must be clearly understood and their interpretation mandates careful consideration on an individual patient's circumstances. Reporting of these results requires caution so that iatrogenic disease or disaster may be avoided.

Human Cytomegalovirus and Epstein-Barr Virus: Double Trouble for Serologists

Paul Austin, Department of Virology & Immunology, LabPlus, Auckland Healthcare Services

Human cytomegalovirus immunoglobulin M (CMV IgM) testing was implemented after an evaluation was conducted in April 1999. A brief description of findings of note from the evaluation will be presented.

Positive CMV DNA results by polymerase chain reaction (PCR) and / or significant changes in CMV serology provided confirmation of CMV infection (true-positive CMV IgM results) in non-neonatal patients. Isolation of CMV from throat or urine specimens in neonates (< 2 months age) was assumed to be consistent with infection. Negative CMV DNA / positive EBV DNA results by PCR and / or unchanging CMV serology was consistent with absence of CMV infection (false-positive CMV IgM results) in non-neonatal patients.

Initial monitoring (4 month period) of assay performance revealed (1) a high reactive rate 62/517 patients tested (12%) and (2) a high proportion of patients with false-positive CMV IgM results (n=20) had dual Epstein-Barr Viral Capsid Antigen IgM (EBV VCA IgM) reactivity [9/13 tested - 69%]. These findings were effectively duplicated in a second larger (n=950) study group, conducted over an 8 month period. Additionally, false-positive EBV VCA IgM reactivity occurred in patients (n=11) with true CMV infection [2/5 tested - 40%].

Usually, patients with true-positive CMV IgM results gave higher optical density (OD) ratios than those with false-positive results, the exceptions to the rule being that if the patients were neonates.

It is our experience that by inspecting patient CMV IgM OD ratio intensities in conjunction with EBV serologic results and the clinical presentation / setting, numbers of likely clinically significant positive CMV IgM results are reduced. This allows clinical staff to focus their attention on obtaining appropriate follow-up specimens for confirmatory purposes. Using this approach we estimate that in our test popu-

lation the incidence of true CMV IgM reactivity is in the order of (1.5%.

Recently Discovered Blood-Borne Viruses

Dr K. Mushahwar, Virus Discovery Group, Abbott Laboratories

Recent advances in the molecular biology field and the introduction of very efficient amplification techniques such as representational difference analysis (RDA) enabled many scientists to identify a variety of human blood-borne viruses. These include GB, TTV, SEN and Sentinel Viruses.

GB virus C (GBV-C) is a flavivirus-like enveloped particle. Its genome is a single-stranded, positive-sense RNA of about 8600 nucleotides that encodes a single large polyprotein consisting of 2844 amino acids. Three major genotypes of GBV have already been identified that correlate with different geographic regions. The predominant routes of transmission of GBV-C appear to be mostly parenteral. Current epidemiologic studies show the presence of GBV-C RNA in many patients and in 2-4% of volunteer blood donors. Most GBV-C infections appear to be asymptomatic, transient, and self-limiting, with slight or no elevation of liver enzyme levels. Most of these subclinical cases resolve after loss of serum GBV-C RNA with a concomitant appearance of antibody to the envelope E2 of GBV-C.

TT virus (TTV) is a recently discovered non-enveloped DNA virus that was initially considered to be one of non-A-GB viruses. Further studies however, have shown TTV to be ubiquitous even among healthy populations casting doubt as to its role in human pathogenesis. TTV is a circular, single-stranded negative-sense DNA genome of approximately 3900 nucleotides in length. At least 3 major genotypes of TTV have been described. Its predominant point of infection is both parenteral and enteric. Besides human, it is also present in high percentage (20-30%) in farm animals such as goats, cows, pigs and chickens. Epidemiological studies show the presence of TTV DNA in 10-90% of volunteer blood donors in various countries.

SENV virus (SENV) appears to be a non-enveloped DNA virus of approximately 3900 nucleotides in length. Details of the circular nature of the virus, its polarity, and single- or double-strandedness is at present not known. SENV encodes for at least 3 open reading frames. Several genotypes of SENV do exist (A-H), each differing by at least 25% in nucleotide sequence. It is claimed that three SENV genotypes, namely, SENV-C, SENV-D, and SENV-H, may be associated with post-transfusion hepatitis. The prevalence of SENV-D and SENV-H in normal blood donors both in Italy and the US is about 2% using the current unoptimised primers and probes.

The Sentinel virus was isolated from sera of some clustered hepatitis cases and appears to be a single-stranded DNA virus consisting of over 3000 nucleotides. Its linear and/or circular nature is at present not known. However, the similarity to TTV at the amino acid level is 30-40% in open reading frame 1. The virus according to its discoverers possesses at least 3 open reading frames. It is found in high proportion in thalassaemic patients, cryptogenic non-A-E hepatitis and blood donors.

At present, there is no evidence whatsoever to show that TTV, SENV and Sentinel viruses are hepatitis viruses. This is based on lack of evidence that (1) these viruses cause either acute or chronic liver disease, (2) that these viruses do not induce hepatitis in naive experimentally infected nonhuman primates with concomitant elevation of liver enzymes and expected abnormal liver pathology, and (3) no proof so far as to the hepatotropic nature of these viruses based on *in situ* hybridisation and immunohistochemical staining studies. These viruses perhaps are recombinant or genotypes of TTV that resulted perhaps from homologous recombination. So far, these viruses cause no known disease in spite of the fact they are present in blood and blood products, hence, the name endosymbionts or orphan viruses or better yet commensal virus.

Laboratory Diagnosis of Whooping Cough by Polymerase Chain Reaction

J.M. Scotter, T.P. Anderson, Microbiology Laboratory, Canterbury Health Laboratories, Christchurch, New Zealand

Whooping cough remains a disease with significant morbidity and mortality. The majority of cases occur in non or partially immunised children, but adults with waning immunity are also at risk. The gold standard for laboratory diagnosis of whooping cough is the isolation of *Bordetella pertussis*, the primary causative agent of whooping cough, from clinical specimens, but this technique suffers from a lack of sensitivity and slow turn-around times.

A recently developed Polymerase Chain Reaction (PCR) assay for the detection of *Bordetella pertussis* has the potential to improve laboratory diagnosis of this disease. The assay involves PCR amplification of a 183 base pair region of the *B. pertussis* genome, and includes an internal positive control to avoid false-negative results.

A preliminary clinical study comparing culture to the PCR for laboratory diagnosis indicates that the PCR shows greatly improved specificity and sensitivity. This will decrease laboratory turn-around times and decrease the number of false-negative or inconclusive results from culture and serological methods.

Molecular Diagnosis of Human Cytomegalovirus (HCMV) Infection and Disease in Transplant Recipients

Hanna Antoszewska, MC Croxson, Virology Department, LabPlus, Auckland Healthcare

Despite remarkable success in surgical techniques and immunosuppressive therapy to prevent rejection in recent years, infection with human CMV remains the major health problem after transplantation.

It affects up to 60% of transplant recipients, causing both mortality and morbidity. The rate of infection and disease depends on type of the transplant with bone marrow recipients having the worst outcome in both.

Molecular biology tests give a substantial improvement over traditional methods (culture or serology), offering both higher sensitivity and shorter time to perform.

Qualitative detection of CMV DNA in peripheral blood cells has provided 100% sensitivity for detection of CMV infection but only 50% or less specificity as a indicator of more serious CMV disease. The assays showing better correlation with CMV disease are: CMV RNA (RT-PCR) and CMV viral load, a quantitative DNA assay (Cobas Amplicor Monitor, Roche).

Measurement of viral load gives an additional tool to monitor treatment and to look for drug resistance. Resistance to ganciclovir (GCV) most commonly used anti-CMV drug has been associated with mutations in the viral UL97 gene.

DNA PCR assays followed by sequencing were developed to detect these mutations.

Applications and pit -falls of above molecular biology based assays are discussed.

Preliminary Evaluation of Polymerase Chain Reaction (PCR) Method for the Diagnosis of Invasive Fungal Infection

WN Patton1, J Scotter2, T Anderson2, L Jennings2, M Schousboe2, W Chan1, J Stevens1, P Ganly1, Departments of Haematology1 and Microbiology2, Canterbury Health Laboratories, Christchurch, New Zealand

Invasive fungal infections (IFI) remain an important cause of morbidity and mortality amongst immunocompromised patients and invasive aspergillosis (IA) has become the leading infectious cause of death following allogeneic bone marrow transplantation. Current methods of diagnosis of IFI are unreliable, prophylactic measures are only partially effective, treatment strategies are usually empirical and available drugs

for IA are potentially toxic or expensive. Improved methods for the diagnosis of IFI are needed and a PCR based approach has the potential to greatly enhance the specificity, sensitivity and speed of diagnosis of IFI and to target anti-fungal therapy more effectively. One such technique has described the detection of fungal pathogens in the blood of selected patients with febrile neutropenia (*J Clin Microbiol* 1997; 35: 1353-60) and we have developed a similar method with design modifications. Extraction of DNA from *Aspergillus* species and contamination issues has proved problematic but the assay is now operable. Preliminary data has shown positive PCR results in two patients (CGL allograft recipient and case of AML) with clinically proven IA (12 out of 27 samples tested), whereas samples have been PCR negative in 10 normal controls and in one autograft recipient without any evidence of fungal infection. In one of these cases of proven IFI samples (n=3) were available two weeks prior to the onset of therapy with systemic amphotericin B (amph B) and these were all PCR positive. The remaining patient tested, a case of myeloma with renal failure and proven aspergillus infection of CAPD fluid, was also PCR positive for IA in blood and CAPD fluids. These preliminary results are most encouraging and data from further testing of a bank of prospectively collected samples of patients with and without proven IFI will be presented.

Advances in PCR Technology

Kevin Barrett, Specialist Services Department, Health Waikato Laboratory, Waikato Hospital, Hamilton

Rapid changes are occurring in the area of Diagnostic Molecular Biology with "Real Time" PCR becoming available which allows detection of PCR products as they are formed. Real-time PCR will lead to very sensitive tests which can be performed in under two hours for infectious agents such as *Legionella* sp. and Herpes simplex virus. Current PCR tests for these agents require six to eight hours to perform, are more prone to problems with amplicon contamination and require much more manual processing. This presentation will look at the Health Waikato Laboratory experience with the LightCycler real time PCR system.

Future Directions and Innovations for PCR

John F Mackay, Molecular Systems, Roche Diagnostics NZ Limited

With molecular testing now an accepted routine in many labs worldwide, the flexibility of methods such as PCR is becoming increasingly developed. The general development is toward increasing automation - for the amplification, detection and the DNA/RNA preparation (as well as integration of all three). Also, by using case-specific genetic markers, more 'individualised' disease therapies are being formed. These trends as well as new diagnostic developments and applications will be described.

COBAS AmpliScreen TM PCR Tests for Blood Screening

Heather Bain, Roche Diagnostics NZ Ltd

The COBAS AmpliScreen PCR System has been developed to directly detect the presence of infectious microorganisms in donor blood by amplifying the nucleic acid sequences specific to the microorganisms. These nucleic acid technology (NAT) blood screening tests offer a much higher level of sensitivity and specificity than routine testing methods currently provide. Despite the current diligent EIA screening of donor blood for the detection of antigens and antibodies, there still remains a residual risk of post transfusion infection for HIV or hepatitis viruses acquired from donors donating in the early window (or latent) period of infection. The power of AmpliScreen is its ability to detect the presence of infection by directly testing for the viral genomic nucleic acids rather than by indirectly testing for the presence of antibodies. An overview of the COBAS AmpliScreen HIV and HCV systems will be presented.

Serologic Determination of the M Type - Should We Continue? The Dilemma of a Reference Laboratory

Martin DR, Sonia Gowan, Streptococcus Reference Laboratory, Invasive Pathogens, Institute of Environmental Science and Research, Porirua

Variability in the N-terminal region of the M protein, encoded by the emm gene, provides the basis for differentiation of streptococci. M serotyping identifies the type-specific epitope on the expressed M protein using type-specific antisera. emm gene sequence typing identifies hypervariable DNA sequences encoding the M serospecificity. The discriminatory power of emm typing approximates that of M serotyping. For most M types there is a one-to-one relationship and M type and emm type are regarded as providing equivalent typing information.

Traditional serotyping methods have been used to determine the M type of Group A streptococci in New Zealand. Reduction in stocks of antisera and the recognition of many newer M types focused attention on the question of continuing to serotype or changing to emm typing. Assessing the value of T typing, Opacity Factor (OF) production, anti-OF typing, determining the emmPCR product restriction digestion pattern (ERP), and emm typing were examined in the context of a Reference Laboratory undertaking surveillance of streptococci causing disease.

M typing, T typing and anti-OF typing, all have similar problems with maintaining stocks of type-specific antisera. Each of these serologic methods has its own unique problems and none typed all streptococci. Comparison of ERP types is not useful when isolates are from diverse sites, times and places. emm typing was the only method that allowed nearly 100% typeability of the isolates tested.

Coughing Up A Storm

Mirjam Horsburgh, Medlab South, Christchurch

Whooping cough continues to be an important disease of infants, children and adults. Most cases occur in children under 6 years of age. Evidence is increasing that Bordetella pertussis infections occur more frequently in older children and adults in vaccinated populations than has been commonly recognised.

In the second half of last year Medlab South saw a significant increase in requests for B. pertussis cultures which has continued up to the present time.

This year from January till June we tested 1898 swabs for whooping cough. A total of 230 (12.1%) specimens were positive. From the 230 positive cultures, 221 strains were identified as B. pertussis, 8 strains were identified as B. parapertussis and 1 strain was identified as B. bronchiseptica.

From the 230 positive cultures we found 30 (13.0%) strains after 7 days incubation and from these 30 strains, 6 (20%) were found after 10 days incubation.

Of the 230 patients with Bordetella infections 125 were 5 years or younger and 105 were 6 years and older.

We are continuing to monitor the number of cases referred to this laboratory.

Extended-spectrum Beta-lactamases Among Urinary Escherichia coli and Klebsiella spp. in New Zealand in 2000

Maggie Brett, Rosemary Stanley, Institute of Environmental Science and Research, Porirua

Antibiotic resistance in gram-negative bacilli due to the action of extended-spectrum beta-lactamases (ESBLs) has been increasing worldwide since the mid 1980s. The organisms may colonise or cause outbreaks, and as the resistance genes are generally carried on plasmids, resistance can spread within and between species.

As little has been published on the incidence of ESBLs in New Zealand, a point prevalence survey was carried out to estimate the rate of occurrence of ESBLs in hospital and community isolates of urinary Escherichia coli and Klebsiella spp. This was done in two parts; survey

A involved laboratories that screened urinary isolates for cephalosporin resistance and submitted resistant isolates for further testing. Survey B involved laboratories that submitted a sample of unscreened isolates for testing.

The minimum inhibitory concentrations (MIC) of the isolates were tested by agar dilution for a range of antibiotics, including cefotaxime, cefotaxime/clavulanic acid, ceftazidime and ceftazidime/clavulanic acid. The presence of ESBL was indicated by a > 2-fold difference in MIC between cefotaxime or ceftazidime with and without clavulanic acid.

From Survey A, the prevalence of ESBLs was determined to be 0.1%; 2 out of 1749 isolates of urinary Escherichia coli and Klebsiella spp isolated in April 2000. No ESBLs were detected in Survey B.

Cost Effectiveness of Screening for Chlamydia in New Zealand

Jenny Dowling, Microbiology Department, Diagnostic Medlab, Auckland

Laboratory tests are considered a cost to the health care system. Appropriate testing may, however, reduce health care costs when treatable conditions are detected and appropriately managed before long-term expensive complications occur. Testing for Chlamydia trachomatis is a useful example because there is evidence that a significant number of infections remain undetected, there are significant private and public health implications and there is a choice of screening strategies.

This paper assesses the cost effectiveness of screening sterile pyuria urine samples of patients, between 15-35 years, who have not previously been tested for Chlamydia. The costs of screening and clinical sequelae are examined using decision-tree analysis. The sensitivity analysis shows the break-even prevalence rates for populations, where the costs of clinical sequelae outweigh the costs of screening.

The Use of Recombinant Proteins in the Development of Enzyme Linked Immunosorbent Assays for Epstein-Barr Serology

William Botelec, Immuno Probe Inc

Recombinant technology has been successfully utilised for the development of many serological ELISA assays. However, just because an antigen is a recombinant it does not necessarily infer that it is suitable for serology. Many problems can occur when using recombinant antigens ie. contamination from the vector, conformational problems, post-translation modification issues etc. Therefore, appropriate functional testing of the protein needs to be performed to determine the performance characteristics of the assays. Since the antibody response is polyclonal in nature, distinct differences can be seen between different antigen preparations. Specific issues regarding the use of recombinant antigens in EBV serology and strategies for assay evaluation will be discussed.

Identification of Common 'Non-dermatophyte' Fungi Seen at the New Zealand Mycology Reference Laboratory

Karen Rogers, Mycology Reference Laboratory, LabPlus, Auckland Healthcare

I will be discussing the identification of the non-dermatophyte fungi seen in New Zealand, which are commonly referred to the Reference Laboratory.

This will include the identification of:

- Zygomycetes
- Black yeast-like fungi
- Fusarium
- Scedosporium
- Scopulariopsis
- Aspergillus
- Acremonium
- Scytalidium (Natrassia)
- Lasiodiplodia

- Onychocola
- Alternaria
- Paecilomyces
- Geosmithia
- Epicoccum
- Stemphylium
- Curvularia
- Bipolaris
- Drechslera

Dermatophyte "look-alikes" such as Geomyces, Beauveria, Auxarthron, Malbranchea, Gymnoascus and Chrysosporium will also be mentioned.

I would like to take this opportunity to discuss the clinical relevance of some of the isolates that are referred for identification, giving some guidelines to aid a laboratory in the evaluation of the significance of the fungal isolate.

I am hoping this will be a fun interactive session and will supply a handout.

Diagnostic Parasitology: A review of current techniques and parasite morphology

Andrew Butcher, Institute of Medical and Veterinary Science, The Queen Elizabeth Hospital, Department of Microbiology and Infectious Diseases, Woodville, South Australia

This workshop will discuss common medically important parasites encountered in routine diagnostic laboratories. The workshop will be designed to expose participants to parasites that could be encountered in a clinical laboratory and will suit both experienced and in-experienced workers. In the first session we will focus on current methods employed in diagnostic parasitology with a detailed discussion on the use of the modified iron haematoxylin stain as a routine screening method for the detection of protozoa. Also discussed will be the suitability of the stain for large and small laboratories along with stain maintenance, quality control, safety and the finer technical points. In the second session a number of case studies will be presented. The clinical features and morphology of each parasite will be discussed to highlight the important diagnostic characteristics. This will be an interactive workshop with audience participation encouraged.

The Dee Vee Tea Party

Murray Smith, Haematology, Medlab Bay of Plenty Limited, Tauranga

Today we will spend examining in minute detail the intricacies of the coagulation pathways. Before we begin we will take a moment to look back at where it all began and to remember that at the heart of all we do is, the patient. Rudolph Virchow and his triad along with a recent case history give us the opportunity to look at the big picture before we dive into the rest of the day's program.

Inherited and Acquired Thrombophilias

Dr Chris Ward, Staff Specialist, Director of Research, Department of Haematology, Royal Northshore Hospital, NSW, Australia

Venous thromboembolism occurring in young patients, patients with a family history of thrombosis or those without a clear precipitating event, should raise the possibility of "thrombophilia". Many novel risk factors have been identified in recent years, using molecular techniques to screen families with inherited thrombosis. Deficiencies of Protein C, Protein S and antithrombin III are rare disorders, accounting for no more than 15% of spontaneous thromboses. An additional 45% of cases can be explained by Factor V Leiden, the prothrombin (PT) 20210A mutation or hyperhomocysteinaemia. Factor V Leiden and PT20210A are common in European populations, with an incidence of 5% and 2% respectively. These are moderate risk factors for thrombosis, but the annual risk increases exponentially in the presence of

other factors or increased oestrogen levels. Many medical disorders result in raised serum homocysteine levels, but simple vitamin replacement can correct moderate elevations and reduce thrombotic risk. Increased plasma levels of Factor VIII, fibrinogen and Factor VIII, fibrinogen and homocysteine levels appear to contribute to arterial as well as venous disease.

Screening for thrombophilic factors in the general population (prior to prescribing the oral contraceptive or surgery) is not cost-effective. Identifying risk factors in patients with venous thromboembolism or their family members may, however, influence the duration of anticoagulation and thromboprophylaxis for at-risk periods in the future. Antiphospholipid antibodies (lupus anticoagulants or anticardiolipin antibodies) are the most important acquired thrombophilic risk factors, and are associated with high rates of recurrence and visceral or cerebral thromboses. Predicting thrombotic risk in asymptomatic individuals with Antiphospholipid antibodies remains a problem for the scientist and clinician.

D-dimers

Louise Montford, Cardiovascular and Clinical Market Support Manager, AGEN Biomedical

D-dimer - the second best Australian invention (after the Hills Hoist - its incredible but true - Australia gave the world the circular clothesline and man are they proud of it!).

The place of D-dimer in acute venous thromboembolism diagnosis and review the five management studies published thus far. Also will supplement by discussing the recent findings of the FACT study - Fibrin Assay Comparison Trial which was sponsored by the ISTH Fibrinogen scientific subcommittee.

The Usefulness of D-dimer Assay in the Diagnosis of Deep Vein Thrombosis

Audrey Grimmer, Haematology, Medlab Central, Palmerston North

Deep Vein Thrombosis (DVT) is a common medical condition where a thrombosis occurs inappropriately in the venous system, usually in the legs. It is important to diagnose and treat to prevent life threatening Pulmonary Embolism. Traditionally diagnosis has been by venography and treatment involved inpatient monitoring of intravenous heparin therapy until warfarin levels became therapeutic.

From April 1999 to April 2000 all patients seen at the Emergency Department at Palmerston North Hospital, with suspected DVT were included in a study to reassess the current procedures for diagnosing and treating DVT patients.

We also evaluated the usefulness of the D-dimer assay in the diagnosis of DVT. Concentrating on two tests, namely the SimpiRED and IL D-dimer assay.

We will discuss the results of these assays and their clinical usefulness.

Heparin Induced Thrombocytopenia

Robin Allen, Haematology Department, Waikato Hospital, Hamilton

Heparin-induced thrombocytopenia (HIT) is a common cause of drug-induced thrombocytopenia, occurring in 2-3% of patients treated with unfractionated heparin. There is a high risk of serious morbidity, particularly venous or arterial thrombotic complications that can be life- or limb threatening. For this reason accurate diagnosis is important. Because these patients often have other conditions that may also cause thrombocytopenia, the clinical diagnosis may be difficult.

Recognition of the pathophysiological underlying HIT has led to a number of diagnostic tests for the disorder, including the platelet aggregation test, the heparin-induced platelet test (HIPA), the 14C-serotonin release assay (SRA) and, more recently, the PF4/heparin ELISA. Each of these assays has its drawbacks. Recently it has been

shown that HIT sera can cause platelet microparticle formation and induce other markers of activation in platelets. This has resulted in the development of sensitive flow cytometric assays for detection of the platelet activation endpoint. These functional assays have several advantages over the traditional tests used for the detection of HIT, including a sensitivity that exceeds that of the SRA.

Thrombophilia Testing

T Day, D Spedding, N Van der Water, Dept. of Haematology, LabPlus Auckland Healthcare

Historically or not too many years ago the focus for screening in the coagulation laboratory was to detect inherited or acquired bleeding disorders.

If we look at more recent history thrombophilia testing has increased in popularity throughout the World as more knowledge attempting to link thrombosis with genetic markers is gathered.

Now that we are aware of the increased risk associated with thrombotic markers and the recently reported deaths of women on the third generation contraceptive pill there has been an exponential rise in the number of requests received in the coagulation laboratories.

The spectrum and complexity of thrombophilia testing has altered with the development of better techniques and new generation analysers.

The advent of protein C activator extracted from *Agkistrodon contortrix* venom and discovery of activated protein C resistance has simplified the testing of protein C, S and APC resistance to a point where they are being performed almost as a routine test in many labs around New Zealand.

Along with the increased range and simplicity of testing comes the change in role of the coagulation technical specialist from ensuring the end point of a global analysis such as PR or APTT is accurate to one of insuring that the results being generated from an analyser and the subsequent interpretation is valid.

This presentation has been prepared to give an insight into the complexity of some of the testing procedures, the pitfalls associated with them and the subsequent difficulties of interpretation.

New Developments in Platelet Structure and Function

Christopher M Ward, Department of Haematology, Royal North Shore Hospital, NSW, Australia

Platelets have a central role in thrombus formation, localising to the site of endothelial cell injury, recruiting other platelets to form aggregates and providing an anionic phospholipid surface for assembly of the tenase and prothrombinase coagulation factor complexes. Key adhesion receptors in platelet adhesion are the von Willebrand Factor (vWF) receptor, the glycoprotein (GP) Ib-V-IX complex, and platelet integrins which bind fibrinogen (IIb/3, GPIIb-IIIa), collagen (2(1) and fibronectin ((5(1).

Platelet activation in response to exposed matrix or external agonists triggers a complex sequence of morphological and biochemical events. Extensive research has focussed on the modulation of receptor function by external and intracellular signalling mechanisms. From this research, specific GPIIb-IIIa antagonists have been developed and used clinically to prevent platelet-mediated arterial thrombosis. Due to their accessibility and ready activation, platelets have become a model system for studying receptor-mediated signalling and cytoskeletal events. In this review, some recent developments in platelet research will be summarised:

1. Platelet interactions with surfaces under conditions of flow, the molecular basis of platelet "rolling", shape change and irreversible adhesion.
2. Mediation of adhesion receptor function by components of the platelet cytoskeleton and signalling molecules.
3. Polymorphisms and mutation of GPIb-V-IX and GPIIb-IIIa and their

effects on platelet structure and function.

4. New insights into platelet function from transgenic mice models.

Several new laboratory instruments have been developed to measure platelet function; these offer a simpler alternative to platelet aggregation studies and skin bleeding times and may have a role in assessing GPIIb-IIIa blockade.

An Evaluation of the Sysmex CA50 Coagulation Analyser

Robert Allan, Haematology, Southern Community Laboratories, Christchurch

This is a presentation on the suitability of the Sysmex CA50 for INR testing at distant satellite laboratories.

The evaluation was performed at our haematology laboratory in Christchurch and included:-

- ease of use
- reproducibility
- comparison of results with the Sysmex CA6000.

It was concluded that the CA50 was a reliable and compact analyser suitable for a low volume laboratory or as a backup for larger analysers.

Cervical Screening - Technology to Enhance Patient Care

Terry Kobler, Business Development Manager Asia Pacific, TriPath Imaging Inc, Australia

With the advent of new technologies, new systems of terminology, larger scale laboratories plus strong government and legal pressures the range of extremely difficult and sometimes expensive QA/QC choices our community faces is greater than ever. Indeed, there is a lack of consensus on exactly how QA/QC methods are to be assessed and enforced.

New technologies within Cytology for the preparation, analysis and reporting of the Pap smear open a new more easily managed QC/QA option.

The standardised preparation of Pap Smears into easily interpreted liquid base smears alleviates many of the quality issues related to preparation, which previously were outside the laboratories control. The smears are now standardised and allow for much easier analysis by humans and computers.

Computer Image Analysis is the next step in maintaining good QC/QA procedures within the cytology laboratory. Not only is the system very consistent and efficient, it lends itself to data collection and data manipulation. These features help generate and maintain records.

Shift Work Problems: Are There Solutions?

Dr Philippa Gander, Sleep/Wake Research Centre, Wellington School of Medicine, Wellington

It is estimated that about 20% of the workforce in developed countries currently work non-standard hours, and this number is increasing. In New Zealand, no nationwide data are collected on the times at which people are working (as opposed to the number of hours being worked). However, annual reviews of collective employment contracts indicate that at least 25% of the total workforce have contracts that include shiftwork provisions. There is extensive scientific evidence that shift work imposes additional demands, compared to regular day work, and that it can have detrimental effects on worker health, safety, and productivity.

At the root of the problem is the fact that shift work runs counter to our innate programming to sleep at night and be active during the day, which is genetically determined and driven by the circadian biological clock in the brain. The circadian clock rarely adapts completely to altered work patterns, because it is constantly being drawn back to its preferred orientation by the unchanged day/night cycle and the activities of the rest of day-active society. The clock also reverts readily to its preferred orientation when people return to being day-active on their

days off.

A person's functioning in the workplace can be compromised in two ways by this conflict between body time and work time. First, they may be trying to work through the daily lowpoint in alertness and performance capacity (mental or physical), which occurs around 2-7 am (slightly later on a night shift). This is when the physiological drive for sleep is greatest. Second, the shiftworker may then be trying to sleep when the body and brain are primed for wakefulness. They can face pressures to cut back on sleep in order to participate in family and social activities, and they may also be trying to sleep when environmental disturbances (light, heat, noise) are greatest. As a result, the restorative values (duration and quality) of shift workers' sleep is often reduced. Inadequate sleep systematically reduces alertness and performance capacity (mental or physical). The effects of several nights of reduced sleep accumulate into a "sleep debt", with sleepiness, performance, and mood becoming progressively worse. Repaying the debt, to restore normal waking function, usually requires two nights of unrestricted sleep. Working at night produces the greatest risk of human error, when the daily lowpoint in performance capacity combines with the greatest likelihood of inadequate sleep. Human errors on the night shift have contributed to a number of major industrial disasters, including the Three Mile Island and Chernobyl nuclear power plant accidents, the grounding of the oil tanker Exxon Valdez, and the explosion of the space shuttle Challenger.

As well as the possible immediate effects of shift work on safety and productivity at work, there is increasing evidence that it can have long-term health consequences for some people. Studies suggest that shift workers, particularly those on rotating shifts, have a higher incidence of sick leave, a higher rate of visits to clinics at the work site, and poorer scores on a variety of indices of health. Sleep complaints, gastrointestinal disorders, and cardiovascular illness have been found to be more common among shift workers (particularly night workers), than among day workers. There is also concern about the effects of shift work on women's reproductive health, particularly in relation to menstrual cycle irregularities and pregnancy outcomes such as miscarriage, low birth weight and preterm births.

Although scientific understanding of these issues has advanced considerably, the development of practical strategies to improve shift work has lagged behind. This paper will review the current scientific understanding of shiftwork problems and describe a comprehensive approach to shiftwork management that includes regulatory initiatives, company policies and procedures, and information on coping strategies for the individual shiftworker. The focus will be on healthcare professionals, and on recent work in New Zealand and Australia.

Two Steps Forward. A Review of Some of the Safety Initiatives Set into Place Over the Last Three Years at Middlemore Hospital Laboratory

Raewyn Bluck, Middlemore Hospital

Safety in a hospital laboratory should encompass more than the identification of hazards in the immediate physical environment of the laboratory. Consideration must also be given to the emotional and physical needs of the staff, patient safety and points of interaction with the rest of the hospital.

Speaking from the point of view of one who not only coordinates the health and safety response for Middlemore Laboratory but also supervises the Central Specimen Reception and Phlebotomy services, the speaker will outline some of the health and safety "responses to need" initiated during the last three years. Topics covered during the lecture will include:

- Safety features incorporated into the design of the laboratory.
- An outline of the function of the Safety committee and its relationship to the lines of responsibility and authority.
- How to get the staff on side (run a safety quiz)?
- A response to needle stick/body fluid incidents.

- The most commonly identified problems (fire alarms, stress from shirt work, chemical management and hand care problems).
- How the laboratory can help with patient safety (the problem of unlabelled bloods, reporting wrist band identification errors, documenting inflamed Luers sites, Phlebotomy training for medical students and nurses, etc).
- Problems encountered along the way.
- Unresolved issues.

It is hoped that the talk will stimulate discussion, and provide a forum for questions and answers.

Going Psycho over Cyclosporines - STILL!

Rebecca Dick, Medlab Kew, Southland Hospital, Invercargill

For patients taking cyclosporine, regular and accurate measurements of the drug levels are an integral part of their treatment. Invercargill lacks a paediatric oncologist so these patients receive the majority of their treatment in Christchurch. Dosage changes of the drug depend on the results obtained from laboratory measurements, therefore it is important that Invercargill measure cyclosporines in a way that correlate with results from Christchurch. This way, there is some continuity of care for patients travelling between the two centres.

Currently this is not the case. Two new cyclosporine methods; the CEDIA homogenous immunoassay from Roche and the EMIT homogenous immunoassay from DADE have been compared with our current TDX fluorescence polarization immunoassay from Abbott. The results were then compared with Canterbury Health Laboratories who use the EMIT assay. Each method had its advantages and disadvantages, which will be outlined in the paper.

With the addition of the Abbott AXSYM chemistry analyser into our laboratory, this paper updates the situation since it was first presented at the Biochemistry Special Interest Group meeting in Nelson earlier this year.

Architecturally Designing our Immunoassay Future

Linda Henderson, Biochemistry Department, Diagnostic Medlab, Auckland

We had five very overworked Abbott AxSYM immunoassay analysers in our department, all in need of some rest and recreation. Our working day had been extended, and the workload seemed never-ending.

The end of May 2000 saw the installation of the first of our two Abbott Architect i2000 immunoassay analysers. The second arrived about a month later.

Initially only thyroid function tests and progesterone were analysed on the Architect i2000, but as patient comparisons were completed and staff trained, more assays came on line. The two Architects perform thyroid function tests (FT4, TSH, and FT3), fertility hormones (LH, FSH, progesterone, proiactin, oestradiol, and (hCG), hepatitis B antibody and surface antigen, and PSA. The remaining AxSYM(s) continue to analyse rubella IgG/M, Toxoplasma IgG/M, CMV IgG/M, therapeutic drugs and troponin I.

How has the Architect i2000 changed our lives? The data analysis, the staff reaction, the effect on the department as a whole. All will be revealed.

Interpretation of Iron Studies

Helen Martin, Deputy Head, Clinical Biochemistry, Gribbles Pathology, South Australia

Iron is a metal essential to human life, occurring in iron containing enzymes and cytochromes as well as in haemoglobin. The mechanisms involved in controlling absorption of iron and maintaining iron balance are still controversial although the sloughing off of unwanted dietary iron in effete intestinal epithelial cells seems to play an important part.

The investigation of iron status crosses the "traditional" boundaries

of pathology disciplines. Uncomplicated iron deficiency is easy to recognise. However, in the presence of infection, inflammation and chronic disease, the diagnosis of iron deficiency by any test, other than the observation of stainable iron in marrow, becomes harder. The measurement of circulating transferrin receptors may be of value in this situation.

At the other end of the spectrum, classical haemochromatosis is again easy to diagnose. However, there are other causes of increased iron stores and of course serum ferritin may be increased with normal iron stores. The discovery of an abnormal gene in the majority of those with haemochromatosis has helped diagnosis and provides an opportunity of predicting the development of the disease before any symptoms occur.

The Effect of Haemolysis on Biochemistry Results

Don Mikkelson, Stephen Tung, Biochemistry Department, Waikato Hospital, Hamilton

Haemoglobin is a very reactive and optically active molecule. Its presence in serum or plasma is indicative of haemolysis and can have profound effects on many biochemical assays. We studied the effects of various levels of haemoglobin in plasma on 52 different Biochemical analyses including immunoassays, up to a plasma haemoglobin concentration of 8g/L. Significant false increases or decreases were seen in 15 assays. 37 assays were not significantly affected by haemolysis.

We recommend that all laboratories measure the level of haemolysis in samples presented for biochemical analyses and place appropriate comments on results demonstrated to be affected by the concentration of haemoglobin in the analysed plasma or serum sample. All samples with plasma haemoglobin above 100 g/L should be rejected as unsuitable for any laboratory investigation.

Recent Advances in Urology

Mr Peter Gilling, Urologist, Promed Urology Limited, Tauranga

There have been a number of advances in each of the major sub-specialities of Urology which have improved the lot of both clinician and patient. These will be covered in brief with an emphasis on pathology and laboratory testing.

Prostate Cancer

Prostate cancer is being increasingly recognised in men with PSA's less than 4(g/L (30% incidence between 2.5(g/L and 4(g/L) but most of these tumours are contained within the prostate. Complexed PSA is less useful than free/total PSA. Human glandular Kallirein 2 (HK2) may be a useful additional marker to PSA. Prostatic intra-epithelial neoplasia (PIN) does not elevate % free PSA or total PSA. Eight to 12 biopsies are better than the standard 6. Modification to surgical and radiotherapy effects are improving patient morbidity.

Bladder Cancer

A number of new diagnostic urine tests have been introduced because of the limitations of urine cytology. The clinical utility of these newer tests has not yet been established with certainty. The Lewis X antigen, Ki-67, E-Cadherin expression, tumour microvessel density and Rb gene expression all correlate with bladder cancer progression. Only p53 positivity (an immunohistochemical technique) however is becoming widely used as a prognostic factor in patients with carcinoma of the bladder. Most patients undergoing radical cystectomy (bladder removal) can safely have an orthotopic diversion (neobladder made of bowel).

Benign Prostatic Hyperplasia

Controversial areas in BPH include prevention of complications (urinary retention, need for surgery) by the use of medical management - particularly Finasteride (Proscar), the use of phytotherapy (natural remedies) and the place of new surgical therapies.

Female Urology

New "conservative" treatments for female incontinence have been introduced such as electromagnetic pelvic floor stimulation and new surgical treatments such as novel "sling" procedures - TVT Tape, bone anchoring and the use of fascial substitutes - have arrived.

Advances in stone disease, reconstructive urology, paediatric urology and other tumours will also be mentioned.

Applied Diabetes Laboratory Management

Dr Steven Morris, Endocrinologist, Pacific Health, Tauranga

Diabetes is experiencing a global epidemic. Present estimates suggest 50% of diabetes is still undiagnosed. Diabetes is more accurately identified as type I (insulin essential) and type II (insulin usually not required).

Type II accounts for the bulk of new and already diagnosed cases. In New Zealand this is strongly influenced by an aging population, increasing obesity rates and increasing relative population percentage of Maori and Polynesian.

Recent modified diagnostic criteria to include a lower fasting plasma glucose will further increase diagnostic rates.

Type I diabetes whilst accounting for only 10-15% of all diabetes has unique and special consideration in the potential diagnosis of other autoimmune linked disease.

The enormous burden of costly treatment, personal and social costs favour early diabetes detection and regular quality glycaemic surveillance to minimise secondary complications. Recently large clinical trials have confirmed the benefits of tight glycaemic control in both type I and type II diabetes. Screening strategies differ with type I detection presently best limited to islet associated antibodies in immediate family members, whereas type II is based on clinical risk.

HbA1C is the recognised Gold Standard but enormous variability exists between different assays and within laboratories.

Other potential useful measures of long-term glycaemic control are being evaluated.

Regular diabetes surveillance requires additional laboratory assistance with a minimum assessment of urine microalbumin, lipids and renal function.

Information presented will link the rationale and process of laboratory tests to their interpretation and clinical applicability.

Antibiotic Resistance in Human Infection - Is it Related to Non-medical Use of Antibiotics?

Dr Timothy Blackmore, Infectious Diseases Physician & Microbiologist, Wellington Hospital

Antibiotics are often the only effective means of treating bacterial infections and also have a role in their prevention. Unfortunately, more and more bacteria are becoming resistant to available antibiotics and the development of new agents is becoming increasingly difficult, expensive and uncommon. There is a real threat that we will lose ground to resistant pathogens and enter what has been described as a "post-antibiotic era".

The general issue of antibiotic resistance will be explored, and the roles of medical use and use of antibiotics in animals will be discussed in relation to the development of resistance.

We have a small human population in NZ surrounded by a large number of meat producing animals. Is human health truly threatened by the use of antibiotics in animals?

Antibiotic Resistance - A Worldwide View

Dr Chris Settle, MB ChB, Specialist Registrar in Microbiology, Leeds United Hospitals NHS Trust, United Kingdom

Resistance to antimicrobial agents is a manifestation of natural selection, and has always been acknowledged as a predictable sequel to

antibiotic use. However, in the last 5-10 years this has started to have an increasingly significant impact on the available therapies for some infections. There is evidence for a link between antibiotic prescribing (at the population level) and resistance development, as well as for spread of resistance between countries/continents (eg. pneumococci). This highlights the point that resistance is fuelled by de novo emergence as well as by spread of strains / genes. Of particular concern is the increase of resistance in *Mycobacterium tuberculosis*, *Staphylococcus aureus*, *Pneumococci*, *Enterococci*, *Enterobacteriaceae* and Gram-negative non-fermenters such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. It is therefore with regard to these organisms that I shall try to summarise the current global problem.

Antibiotic Resistance: How It Might Affect Your Doctor's Prescription

Dr Selwyn Lang, Clinical Microbiologist/Infectious Disease Physician, Middlemore Hospital/Diagnostic Medlab, Auckland

Escherichia coli, the most common cause of urinary tract infections, and *Staphylococcus aureus*, the cause of boils, other skin and soft tissue infections, septic arthritis and osteomyelitis, are each isolated in the clinical laboratory about 10x as often as any other bacterial pathogen. *Streptococcus pyogenes*, which cause pharyngitis, impetigo and cellulitis and which may lead to rheumatic fever and to glomerulonephritis, is a distant third, followed by *Haemophilus influenzae* and *Streptococcus pneumoniae*, which cause respiratory infections, sinusitis and otitis media and *Staphylococcus saprophyticus*, which is almost exclusively a cause of urinary tract infections. Other significant bacterial isolates are relatively uncommon, although *Chlamydia trachomatis* is identified in about 4000 patients in Auckland each year (more often than *H. influenzae*).

For the past decade the proportion of *E. coli* isolates resistant to amoxicillin appear to have reached a plateau at about 50%. Recently there has been an increase in resistance to trimethoprim (~20%) and to amoxicillin/clavulanate (~4% in the community but up to 30% in some hospitals). Almost all remain susceptible to nitrofurantoin, cephalosporins, fluorquinolones and gentamicin. Extended spectrum beta-lactamase producers are uncommon.

The proportion of methicillin-resistant *S. aureus* (MRSA) varies widely depending on the ethnicity of the population. The most commonly isolated strains appear to have originated in the Western Samoan population. These are seldom multi-resistant and spread primarily in the community rather than being hospital-acquired. There has been a dramatic increase in resistance to mupirocin since it became available 10 years ago and to a lesser extent to fusidic acid. We have yet to encounter strains with reduced susceptibility to vancomycin.

S. pyogenes resistant to macrolides is uncommon.

Penicillin-resistant *S. pneumoniae* (PRSP), which are often multi-resistant, have become common (~30%) in the past 5 years. Of 376 isolates causing invasive disease during 1998, 15% were penicillin non-susceptible: about half of these being resistant (MIC(2mg/L) and half intermediate resistance (MIC 0.12-1 mg/L). 0.5% and 6.1% of invasive pneumococcal isolates were respectively resistant or of intermediate resistance to the third generation cephalosporins, cefotaxime and ceftriaxone. With the exception of pneumococcal meningitis, however, penicillin in high doses, or oral amoxicillin, remains the treatment of choice for pneumococcal infections due to so-called penicillin-resistant strains.

15-25% of *H. influenzae* are beta-lactamase producers and resistant to amoxicillin. A similar proportion is resistant to cotrimoxazole. Amoxicillin/clavulanate-resistant isolates are very seldom encountered and resistance to cefaclor and to tetracycline is also uncommon.

S. saprophyticus susceptibilities are difficult to interpret: many appear resistant to both penicillin (or amoxicillin) and methicillin using the conventional break-points for staphylococcal susceptibility testing, however only a small proportion have high levels of resistance and

amoxicillin or other antibiotics usually used for the treatment of urinary tract infections are usually effective.

How Should We Best Detect Antibiotic Resistant Organisms?

Dr Chris Settle, MB ChB, Specialist Registrar in Microbiology, Leeds United Hospitals NHS Trust, United Kingdom

The current increase in resistance to antimicrobial agents of a number of bacterial species is a matter of great concern amongst health care professionals. Some of the more serious problems have been highlighted in an earlier presentation, and now I would like to suggest how we can best try to detect such resistant pathogens in the laboratory. In doing so, I shall outline some of the current methods, both old and new, for detecting MRSA, VISA, VRE, penicillin resistant *Pneumococci*, ESBL producing Gram-negative bacilli and MDRTB. I shall consider standard versus automated detection methods and also whether we should be looking for resistant strains alone or for evidence that resistance is developing (shift or drift)?

The Use of Honey as an Antimicrobial Agent

Prof Peter Molan, Director of Honey Research Unit, University of Waikato, Hamilton

There has been a renaissance in the medical usage of honey as its potential as an antimicrobial agent is becoming recognised, particularly in the treatment of wounds and in ophthalmology. A clinical trial has also shown its effectiveness for the treatment of bacterial diarrhoea. But although the ancient physicians were aware of differences in the therapeutic value of different honeys, in almost all modern reports honey is referred to generically, despite microbiological studies having shown more than 100-fold differences in the potency of the antibacterial activity of various honeys.

The antibacterial activity of honey is due mostly to enzymatically generated hydrogen peroxide, but there are some nectar sources, manuka outstandingly, that provide additional antibacterial components. As catalase in body tissues and serum will inactivate at least some of the hydrogen peroxide, the extra activity in manuka honey may be very significant clinically.

Various clinically important species of microorganisms have been tested for their sensitivity to the both types of antimicrobial activity in honey, using standardised honeys of mid-range antibacterial potency (as are available commercially). The minimum inhibitory concentrations of honey (% v/v) found for bacteria isolated from infected wounds, for manuka honey and for pasture honey, respectively, were: 58 strains of coagulase-positive *Staphylococcus aureus* 2%-3% & 3%-4%; 20 strains of *Pseudomonas*, 5.5%-8.7% & 5.8%-9.0%; 16 strains of (-haemolytic streptococci 4.5%-9.7% & 5.3%-9.8%; 82 strains of MRSA, 4%-7% & 3%-7%; 56 strains of VRE, 5%-10% & 8%-20%; 5 strains of *Acinetobacter baumannii*, 6%-8% & 7%; 4 strains of *Stenotrophomonas maltophilia*, 3%-4% & 4%-6%. For 12 common gastroenteritis-causing species of bacteria the MICs were 2%-11% & 3%-8%.

Antibacterial Activity of Manuka Oil

NG Porter1, BA Peddie2, K Randall2, ST Chambers2, 1 New Zealand Institute for Crop & Food Research Ltd, Lincoln, 2 Christchurch Public Hospital, Christchurch

This paper summarises recent results demonstrating control of Gram-positive bacteria by essential oil of manuka. Commercial manuka oil (Manex™, 25% α -triketones) and a 98% α -triketone concentrate were compared with other manuka, kanuka and Australian tea tree oils, using liquid broth and agar plate dilution series, and agar plate inhibition zone tests. Antibacterial activity of manuka oil is clearly associated with the presence of α -triketones. For *Staphylococcus aureus*, *S. epidermidis*, *S. saprophyticus* and *Enterococcus faecalis*, minimum

inhibitory concentrations (MIC) of Manex oil were consistently lower (0.016 - 0.063% (v/v)) than Australian tea tree (>0.25%). Minimum bactericidal concentration (MBC) values for Manex oil and the α -triketones against these bacteria were close to the MIC values, indicating useful bactericidal activity. MIC values for Manex oil and α -triketones against Methicillin-resistant *S. aureus* were the same as for susceptible *S. aureus*, indicating a different mechanism of action. Current work on kill curves will be summarised. Similar results were obtained from liquid and agar dilution series for all oils. Practical problems (zone distortion and instability of oil dispersion) were experienced with inhibition zone tests and results were less consistent. Gas chromatographic analysis showed that Mueller-Hinton agar prevented non-polar hydrocarbon diffusion but allowed polar oxygenated component diffusion from the point of application of the oils. The zone diameter may be affected by the oil composition and the diffusion process and is therefore an unsuitable parameter for simple comparisons of efficacy of essential oils that have different chemical compositions and especially different balances of polar and non-polar compounds.

Blood in a Minute

Sheryl Khull, Service Leader, New Zealand Blood Service Manawatu, Palmerston North Hospital

Medical and surgical emergencies can make impossible demands on the blood bank. The trauma team would be happy if we could provide blood in a minute (or less). At Palmerston North Hospital, we discussed the various needs and restrictions of the trauma team and the blood bank, and then developed a plan to provide blood, which is as safe as possible within the available time - even when there is no time. The range of services offered includes fully cross-matched blood (safest); spin cross-matched blood; group specific uncross-matched blood; emergency O Negative blood held in theatre's blood fridge; or the Emergency Transfusion Pack, comprising six units of group O blood delivered to the patient. This solution may not work everywhere, but what can and should work everywhere is the open and effective communication between blood bank and trauma team, both while planning services and during emergencies.

Transfusion Medicine - A Surgeon's Perspective

Chris Holdaway, Vascular Surgeon, Waikato Hospital, New Zealand Army Surgeon

This paper will briefly discuss Perioperative Haemorrhagic Disorders, including those related to Platelet factors, Coagulation factors, Environmental factors including Hypothermia, Technical factors and iatrogenic contributions. It will do so within a Vascular Surgery / Trauma background.

The paper will then explore Transfusion Therapy from a Vascular Surgical perspective. Transfusion therapy will be considered from the simplistic Operation Room classification of RBC Transfusion, Platelet Transfusion, and Transfusion of Coagulation Factors.

An overview of clinically significant complications of Blood Transfusion will be covered, under the headings of Immunological and Infective.

Finally Technical Advances in the Vascular Surgical and Anaesthetic fields will be discussed, in relation to their Transfusion saving contributions, including Preop Autologous Blood Collection, Cell Saving Devices, Rapid Infusion Devices, Improved Graft Technology, and Endovascular Surgery.

Blood Transfusion-An Anaesthetists Perspective

David Laidlow, Anaesthetist, Lakeland Health, Rotorua

Uncontrolled haemorrhage is, at best, disconcerting, but is often frankly terrifying to all the staff involved. During normal working hours in the smaller peripheral hospitals the situation will usually be brought

under control, but inevitably trauma occurs out of normal working hours, when the reduction in staff numbers "on the shop floor" poses further logistical problems, which may well have a bearing on the final outcome.

In addition, smaller hospitals carry less blood and blood derivatives than larger centres, so that "ideal" blood replacement therapies are not necessarily possible. Under these circumstances, it is the presenters belief that nothing is as effective in major haemorrhage as fresh whole blood, which is now far less available since the advent of fractionated blood products.

Antenatal and Postnatal Forum - Session 1

This forum is not intended as purely a sit and listen lecture. It will be a mix of prepared information presented to participants, dry workshop exercises and group discussions.

Laboratory Focus

Grant Bush, Charge Technologist, Transfusion Science, Medlab Bay of Plenty

Antenatal Testing

- Group & Screen
 - What stage of pregnancy to test
 - Du testing (or not)
 - What happens if screen is positive
 - How Immunisation Occurs (covered in more detail in clinical session)
- Antibody Identification
 - Panel Interpretation (covered in detail under postnatal testing)
 - Methods used & Significance ie. IAT vs Enzyme / desirable characteristics of screening cells
 - Which antibodies are clinically significant & why (covered in more detail under monitoring of HDN)

Sheryl Khull - Service Leader, NZBS - Manawatu

- Antibody Titers
 - Methods available eg. Tube vs Gel, Albumin
 - Discussion with audience on methods used in labs throughout NZ
 - Significance of results
- Paternal Testing

Mike Geurts - Team Leader, NZBS - Waikato Blood Bank

Postnatal Testing

- Group & Screen vs Group & DAT
 - Effect of a positive DAT on results - blocking antibodies, IAT typing
- Antibody Identification
 - How to read panels / some examples to work through
- Antigen Typing
- Eluate
 - Methods available
 - Significance of Serum & Eluate results
- Crossmatching
 - Top-up transfusions
 - Exchange transfusions

Antenatal and Postnatal Forum - Session 2

Clinical Focus - Open to Lab Staff, Midwives & GP's

Geoff Herd - Charge Scientist, Blood Bank, Whangarei Hospital

Prevention of HDN

- How immunisation occurs - difference between ABO & other blood group antibodies
- Prevention of Anti-D formation
 - When to administer - timing & patient categories
 - Brief overview of commercial Anti-D production
 - Anti-D preparations available & dosage

- Significance of Kleihauer results
- Use of flow cytometry
- Prevention of other antibodies causing HDN
- No prophylactic treatment
- Predicting effect based on fathers phenotype

The session is intended as an open forum for laboratory staff, general practitioners and midwives on the prevention of haemolytic disease of the newborn.

The topics covered will include how maternal immunisation occurs in ABO and Rh haemolytic disease, the use of anti-D prophylaxis for the prevention of Rh(D) haemolytic disease and the methods used to estimate the extent of foetal maternal haemorrhage. A brief overview of haemolytic disease caused by other antibodies will be given.

Dr Susanta Ghosh - Transfusion Medical Specialist, NZBS - Waikato

Monitoring of HDN

- Clinical monitoring
- Which antibodies are clinically significant & why
- Laboratory testing
- Antibody titers
- Amniocentesis
- Bilirubin
- Consequences of HDN

Treatment of HDN

- Antenatal treatment
- Postnatal treatment
- Advice given to prospective parents

Pulsotyping - A Microbiological Tool for the Noughties

S Fenwick, M Leyland, K Walker; N Boxall, S Mannering, J Connoll, PFGE Typing Laboratory, Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North*

Sub-typing of bacterial species has been performed for many years for surveillance and epidemiological purposes and has traditionally relied on sero-typing or phage-typing, both of which are usually carried out at reference laboratories. The advent of molecular typing techniques provided an alternative to these methods, and sub-typing of bacteria can now be easily carried out in any laboratory having the required equipment. Of the many new techniques that have been developed, pulsed-field gel electrophoresis of bacterial macro-restriction fragments (PFGE) has become increasingly used by laboratories in many countries to subtype a wide range of bacterial species. The technique is generally accepted to be robust, reproducible and reliable and, if standardised protocols are used, laboratories can compare or share results electronically. We have been using PFGE for four years and have adapted the technique to investigate a diverse range of bacteria. In addition, we are currently attempting to establish a network of laboratories using PFGE for surveillance of enteric pathogens, in particular *Campylobacter jejuni*, and similar networks have been established in the USA and EEC. A brief overview of the technique, equipment, costs and pitfalls of PFGE will be given. Results from studies performed using PFGE with a number of genera, including *Campylobacter*, *Salmonella*, *Serratia*, *Brucella* and *Staphylococcus*, will be used to illustrate the practical applications of PFGE and the epidemiological questions that we have been able to answer. These include, outbreak investigation, tracing sources of infection, investigating zoonotic transmission of pathogens, identifying the range and spread of bacterial subtypes in farming situations, investigation of potential vaccine breakdowns, potential global spread of infectious agents and monitoring of hygiene procedures in slaughterhouses.

***Campylobacter upsaliensis/helveticus* in CATs and dogs**

Karen Cooper, Auckland Animal Health Laboratory, Agriquality New Zealand

Campylobacter upsaliensis is a thermophilic campylobacter that can cause enteritis in animals and humans. *C. helveticus* is also a thermophilic campylobacter, however it is isolated more frequently from healthy animals than from those with enteritis. Skirrow's agar is one of the most common media used for the isolation of *Campylobacter* spp. The growth of these organisms is however inhibited by the antibiotics present in Skirrow's agar, so they are seldom encountered in clinical laboratories. The low isolation rate of these organisms may also be due to too short an incubation time. Literature has suggested that an incubation of up to eight days is essential for the isolation of *C. upsaliensis*.

CAT agar (Cefaperazone, Amphotericin, Teicoplanin agar) is an alternative campylobacter isolation medium that had not been previously used in our laboratory. CAT agar was used in parallel with Skirrow's agar for a six month period to determine if it was a suitable media for the isolation of enteric *Campylobacter* spp. The duration of culture was lengthened to one week to determine what is the ideal incubation time for these organisms. All campylobacter isolates were identified using routine biochemical tests including, Gram's stain, oxidase, catalase, susceptibility to cephalothin and naladixic acid and hippurate hydrolysis.

Apart from the *C. upsaliensis/helveticus* isolates (which did not grow on Skirrow's agar) we found that several *C. jejuni/coli* isolates grew only on CAT agar and not on Skirrow's. Half of the *Campylobacter* isolates grew within three days of culture and the other half grew after three days incubation.

Failure to isolate *C. upsaliensis/helveticus* was undoubtedly related to the use of unsuitable isolation procedures, primarily inhibitory media and too short an incubation time.

Salmonella Brandenburg - A Woolly Problem

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S. Brandenburg is an infrequently occurring serotype worldwide, and in New Zealand represented 1.2% of 16,887 salmonellae isolated 1985-1996. Non-human isolates over the same time period included occasional isolates from pigs, cattle, poultry, meat and bone meal, and a dog.

In 1996 *S. Brandenburg* was isolated from a ewe in mid-Canterbury, where a number of sheep abortions and subsequent ewe deaths were noted.

In 1997 isolates were received from Southland sheep and in 1998 from Otago. A similar pattern emerged during the lambing season in these areas in 1999. A concurrent rise in human isolations, particularly in farmers and agricultural workers over the same time period, was also observed.

Expect the Unexpected

Viv Quayle, Auckland Animal Health Laboratory, Agriquality New Zealand

The laboratory routinely runs albumin estimations on a range of animal species using a Hitachi 912 analyser. The introduction by Roche diagnostics of a replacement albumin method required a routine comparison of the two methods.

Roche Albumin and Roche Albumin Plus estimations; both utilising bromocresol green; were performed on 42 patient samples selected randomly over a period of 4 weeks. A small group of outliers was observed; these were identified as equine samples.

A subsequent investigation of over 200 samples including feline, canine, equine canine and bovine sera showed species specific differences in the comparisons. The average difference for small animals was

significantly higher, whereas large animals were slightly lower. These differences flow on to calculated tests; specifically globulin & albumin/globulin ratio.

As the original reagent is no longer available a project to set new feline and canine references ranges was deemed necessary.

The Affects of Anticoagulants on Ferroxidase, Glutathione Peroxidase and Vitamin B12 Testing

Mariette Komene, Animal Health laboratory, Ruakura

Ferroxidase, Glutathione peroxidase, and Vitamin B12 are tests that are regularly requested to determine Copper, Selenium and Cobalt levels in production animals. All of the reference ranges and production response levels have been based on serum levels for Ferroxidase and Vitamin B12 and whole blood EDTA for Glutathione peroxidase. This makes these tests limited on having the correct specimen type being taken.

To remove these limitations samples using the anticoagulants EDTA and Heparin were looked at to determine if they were suitable sample types. Samples of plain serum, EDTA and Heparin whole blood were taken from each animal. The serum and plasma from each tube were separated and refrigerated. A portion of the whole blood was kept before the plasma was removed. In most instances analysis of samples took place the next working day. All results were then compared.

For Ferroxidase results from 44 animals were obtained. Results showed that Heparin plasma was a suitable sample type to determine Ferroxidase levels but reference levels would need to be determined. EDTA plasma was found to be unsuitable to test for Ferroxidase as the EDTA appeared to affect the test method and the results were unusable.

For Glutathione peroxidase results from 30 animals were obtained. Heparin whole blood can be used to determine Glutathione peroxidase levels.

For Vitamin B12 results from 60 animals were obtained. Preliminary findings show that both EDTA and Heparin plasma may be suitable sample types to determine Vitamin B12 levels however the EDTA plasma results were lower than serum results and the Heparin plasma results were higher.

More work will need to be done to determine whether these differences require separate reference ranges.

Paediatric Haematology Neonatal White Cell Morphology Workshop (Dry) Diagnostic Approach to White Cell Disorders in the Neonate

Dr Robyn Rodwell, Haematology Division, Mater Hospitals, South Brisbane, Australia

The diagnosis of white blood cell (WBC) disorders in the neonate may be difficult due to the numerous peripartum factors that may influence WBC values. The newborn infant is immunocompromised compared to adults with profound differences in neutrophil numbers and functions. The transition from intrauterine to extra-uterine life is associated with rapid changes in WBC kinetics and an increased susceptibility to bacterial infection. In the first days of life, the majority of full blood counts (FBC) are performed to exclude infection. A systematic approach based initially on maternal and infant history, the presence or absence of risk factors for infection and the presence or absence of congenital abnormalities is required. The use of standardised criteria for the classification of neutrophilic forms and reporting of "toxic changes" and their use in a computer generated haematologic scoring system improves the diagnostic value of the FBC as a screening test for sepsis. Neutropenia and/or thrombocytopenia may result from maternal pregnancy-induced hypertension, intrauterine growth retardation, infection, or be of immune (passive immune secondary to maternal autoimmune disease or alloimmune due to in utero maternal/foetal interactions) origin. Congenital viral infections such as cytomegalovirus

may cause a leuko-erthroblastic blood film. Exaggerated leukocytosis with occasional blasts may result from in utero infection. Occasionally, haematologic diseases such as transient abnormal myelopoiesis, which is associated with Trisomy 21, or congenital leukaemia may manifest in the midst of these development processes. A systematic approach simplifies the diagnosis of WBC disorders in the neonatal period.

Paediatric Haematology Neonatal Red Cell Morphology Workshop (Dry) Red Blood Cell (RBC) Morphology and RBC Disorders in the Neonate

Dr Robyn Rodwell, Haematology Division, Mater Hospitals, South Brisbane, Australia

Recognition of abnormal red blood cell (RBC) morphology and RBC disorders of the neonate requires knowledge of normal RBC values in the neonatal period, the RBC morphology changes that occur in the healthy newborn infant and of specific RBC disorders that may become apparent in the neonatal period. Compared to the healthy adult, the RBCs of newborn infants are macrocytic. Increased polychromasia and small numbers of poikilocytes, pyknocytes and spherocytes may be seen in the healthy infant. Anaemia on the first day of life is most commonly due to fetomaternal haemorrhage, twin to twin haemorrhage, haemolytic disease of the newborn, disseminated intravascular coagulation associated with systemic bacterial infection or in-utero viral infections. Occasionally it may be associated with Hb Barts hydrops or the manifestation of haematologic disease such as congenital leukaemia. Premature infants in intensive care units may subsequently become anaemic due to iatrogenic blood loss associated with frequent blood sampling. On the first day of life spherocytes may be seen in infants with congenital cytomegalovirus infection who usually show a leucoerythroblastic blood film; marked spherocytosis may be due to ABO haemolytic disease or hereditary spherocytosis. Additional causes of spherocytosis, which usually manifest after the first day of life include G-6PD deficiency, T cryptantigen activation and polyagglutinability (associated with necrotizing enterocolitis and infusion of standard plasma-containing blood products) and clostridial infections. The presence of fragmented cells, pyknocytes and Heinz bodies may alert to oxidative haemolysis, the cause of which is usually difficult if not impossible to elucidate. The characteristic features of RBC membrane disorders such as hereditary elliptocytosis or hereditary ellipto-stomatocytosis may be apparent on the first day of life or manifest within the first weeks of life. Anaemia of prematurity is a physiologic phenomenon, which is seen in developing premature infants.

Electronic Integration of Laboratory with Client

Dr Harry Pert, GP, Ranolf Medical Centre, Rotorua

Enormous changes have taken place in the structures of General Practice over the last decade, with the vast majority of GPs now being members of Independent Practitioner Associations. With this change has also come a change in the role of General Practice, with Practices and the Practice Organisations taking greater responsibility for the care of populations, as well as the traditional focus on individual health care.

These changes are reinforced by new contracts requiring a greater accountability for both health outcomes and the responsible use of referred services including laboratory investigations.

This presentation will outline some of the changes in recent years from a General Practitioner's perspective, and will outline in more detail some of these issues and the needs of General Practice for the next few years. The changing role of general practice, the emergence of the IPA movement and the opportunities of Information Technologies will be explored, with particular emphasis on the importance of Laboratory involvement.

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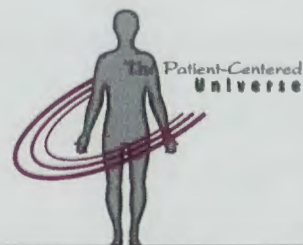
HOSPITAL BENEFIT

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- Rapid results—for fast clinical decisions
- Prevents need for repeat samples due to testing delay

- No hidden costs
- Eliminates additional, costly supplies such as heparin syringes
- Simplifies QC—saves time
- Minimizes maintenance costs

- Allows POC coordinator to track cartridge usage—for more accurate inventory management



A B B O T T
Point-of-Care

Drugs in the Workplace

Ron Couch, LabPlus, Auckland Healthcare

In New Zealand in 1987 a Minister of Health for the Labour Government initiated the term "preventative medicine" to include the testing of employees for drug abuse. The National Institute of Drug Abuse (NIDA) convened "a landmark" conference in 1986 to discuss and achieve consensus on drug-testing issues. The Institute for a Drug-Free Workplace (Washington) found in a survey an increasing positive attitude to testing. In 1995, 71% of employee respondents indicated that their company had a drug testing policy compared to 59% in 1989.

NIDA proposed testing for the following five, "NIDA-5", groups of drugs. Cannabinoids, cocaine, amphetamines, opiates and phencyclidine. An expanded test regime includes benzodiazepines, barbiturates, methadone, methaqualone, propoxyphene and ethanol. The NIDA "Guidelines" for testing have as a cornerstone the "two test" concept: an initial is performed on each class of drugs, and if positive, a confirmational test using a different technology. Specifically, the initial testing technology is immunoassay, and the confirmatory testing technology is gas chromatography-mass spectrometry as the required standard of practise in workplace testing.

A urine specimen is used for such testing since its collection is considered to be the least invasive, and this body fluid offers a mirror of drug intake over a recent time period. Certain cut-off concentrations have been accepted globally above and below which a drug or drug group would be reported as detected or not detected.

Donors may attempt to avoid drug detection by supplying a specimen that is not their own or they may dilute the specimen in-vivo/in-vitro. The witness employs various techniques such as a temperature determination 2 minutes after voiding. Dilution can be ascertained using a creatinine assay. A donor may adulterate the specimen using household products such as bleach, vinegar, detergents, salt or ascorbic acid. "Cottage industries" may sell adulterants such as gluteraldehyde and most recently pyridinium chlorochromate marketed as "Urine-Luck". The transfer of the specimen, in sealed containers, from the collection site to the laboratory is detailed with a Chain of Custody Request Form. Further, a laboratory carrying out such analyses must be security separated from other specialities in a larger laboratory. Companies supplying kits for immunoassay tests have addressed the issue of cross-reactivity and false positive results by using more specific mono-clonal antibodies. However, experience shows that the opiate immunoassay will detect the anti-tussive, pholcodine and also an ingredient in poppy seed cakes where the seed may be from a *Meconopsis* species rather than *Papaver somniferum*. Further our NZ "home-bake" industry supplies clandestine mixtures of heroin, 6-monoacetylmorphine, morphine and codeine. A false amphetamine is often produced by the metabolites of prescribed drugs such as chlorpromazine, ranitidine and phentermine. Many drugs and their metabolites are excreted as glucuronide or sulphate conjugates. The immunoassay kits may or may not have suitable cross-reactivity to such conjugates. False negative benzodiazepines tests are possible if an initial hydrolysis is not carried out.

The interpretation of results often involves an understanding of the pharmacokinetics associated with the drug. For example, tetrahydrocannabinol is known to be rapidly cleared from highly-perfused tissues but then sequestered for much longer times in fat stores. Depending on drug use, a donor's urine may be positive for cannabinoids for only 24 hours or up to 10 weeks after abstaining.

Workplace drug testing will involve the analyst in court proceedings. Such presentations require knowledge of analytical techniques and pharmacokinetics. Such an Employment Tribunal court proceeding will be described.

Legal Aspects of Drug Testing in the Workplace

Monique Pinsoneault, Solicitor, Legal Department, Employers and Manufacturers Association, Auckland

There are a number of reasons for which an employer may consider it desirable or necessary to test whether current and prospective employees are using or have used drugs. Such reasons include:

- to screen applicants for drug use;
 - to protect the safety of employees and others;
 - to assist in accident prevention;
 - to assist in accident investigation;
 - to ensure appropriate levels of performance and production are being maintained;
 - to ascertain whether there has been compliance with an employer's code of conduct or employment contract in relation to drug use.
- Whether such tests are lawfully able to be administered in New Zealand is not entirely certain and employers need to exercise extreme care with this issue. The most likely situations which will give rise to other judicial pronouncements on drug testing will be:
- a challenge by employees to the introduction of a drug testing policy by the employer;
 - an alleged unjustified dismissal of a person who has been dismissed after testing positive for drugs or who refused to undergo a drug test;
 - an alleged unjustified disadvantage following the giving of a warning to a person who tested positive for drugs or who refused to undergo a drug test;
 - a claim by an employee that a provision in his/her contract of employment relating to drug testing is harsh and oppressive.

Each scenario would raise a number of issues and may attract significant financial consequences. Any legal challenge to drug testing would likely result in the employer having to establish the accuracy and reliability of the procedures and methods used for drug testing, as well as their compliance with relevant legislation.

An overview of the legal aspects of drug testing requires an examination of a number of statutes including the Health and Safety in Employment Act 1992, the Human Rights Act 1993, the Bill of Rights Act 1990 and the Privacy Act 1993 and my paper will deal with these.

Cervical Screening - Technology to Enhance Patient Care Clinical Focus

Terry Kobler, Business Development Manager Asia Pacific, TriPath Imaging Inc, Australia

The late 1990's saw the merging of three companies working within the cytology field into one company called TriPath Imaging Inc. The merging of these technologies has increased dramatically the potential of automated cytology to deliver an integrated solution involving preparation, primary screening and telepathology. The cytology lab of tomorrow will use these tools to enhance the quality of the screen service it provides to the community.

TriPath Imaging has developed a range of products which when used together automated the entire process from preparation, screening through to reporting and data management.

The liquid based Pap smear is completely automated with the AutoCyte Prep system. The system is a batch-oriented robot, which prepares and stains 48 samples at a time. The process is FDA approved to replace the conventional Pap smear and prepares a homogeneous 13mm diameter circle of cells representing the sample collected. All obscuring artefacts are removed and the diagnostic cells are enhanced.

The Primary screening system is specialised for mass screening and archiving of normal samples without human review. The system also locates the most abnormal areas on the smear for human interpretation. The system can screen both conventional and liquid based smears. The system is FDA approved for primary screen of Pap smears.

The final tool available allows for the integration of the entire

process. Software has been developed to allow for the management of the systems and the sharing of information within the laboratory and between laboratories. Telepathology could then be used to enhance the laboratory service and allow for the sharing and monitoring of standards throughout the country.

A Survey of Intestinal Parasites in Timorese Refugee Children

Wayne Melrose, D Yap, G Coleyer, School of Public Health and Tropical Medicine, James Cook University, Townsville, Australia

Faecal samples were collected from 111 Timorese refugee children accommodated in a "safe haven" in Victoria. Forty five per cent of the samples contained one or more helminth or protozoan parasite. A highlight was the discovery of a case of *Hymenolepis diminuta*, a rare parasite in humans.

Recognising Fantasy: Gamma-Hydroxybutyric Acid Analysis and a Poly-abuse Report

Dr Ron Couch, Chemical Pathology, LabPlus, Auckland Healthcare

A 28-year-old female who had been unrousable for 3 hours was presented to emergency. The initial Glasgow Coma Score was 9(E3M5V1), which later dropped to 6(E2M3V1). Other features were essentially normal. Her colleagues reported the use of gamma-hydroxybutyric acid (GHB).

GHB and an internal standard cyclohexanecarboxylic acid were isolated using a liquid-liquid extraction procedure. They were then converted to their trimethylsilylether derivatives. Gas chromatography on a DB-1 capillary column and flame ionization detection gave peaks for the derivatives of GHB and cyclohexanecarboxylic acid and also an external standard (tetradecane) at 12.80, 12.45 and 15.64 respectively. Gas chromatography-mass spectrometry on a HP-5MS column identified GHB-diTMS with retention time 7.9 min and characteristic fragment ions 147,73,117,233 and 204.

The specimen was positive for amphetamines by immunoassay. Gas chromatography with nitrogen phosphorous detection on a HP-1 capillary column gave peaks with retention times of 2.29 and 2.07 minutes identified as methamphetamine and amphetamine respectively.

Other analytical techniques of GHB analysis require acid catalysis to gamma-butyrolactone. The method described is specific for GHB. GHB should be considered in cases of unexplained coma as GHB overdose will not be detected by routine toxicological screening.

The Use of EHEC Medium for Isolating STEC (E coli) from Food Enrichments

JA Hudson¹, C Nicol², J Capill¹, J Bennett², 1 ESR Ltd, Christchurch Science Centre, Christchurch, 2 ESR Ltd, Kenepuru Science Centre, Porirua

Numerous methods exist for the detection of *Escherichia coli* O157:H7 in foods, but methods for the detection of other shiga toxin-producing *E. coli* (STEC) are not well developed. One such medium, Enterohaemorrhagic *E. coli* (EHEC) agar, was evaluated for its ability to recover serotypes O157:H7, O26 and O113:H21 from raw mince, pasteurised milk and salami. The method detected around 1 cfu in 25 ml of milk, but was less sensitive with salami, requiring 10-1,000 cfu 25 g⁻¹ (depending on serotype) for detection. Recovery was easily achieved with cooked meats. Recovery from raw minced meat was difficult, but sometimes possible. Testing more presumptive colonies than were tested in this study would presumably increase the sensitivity of the method.

Whole Blood Analysis - The Good, The Bad and The Jaundiced

M Wise, C L Harvey, LabPlus, Auckland Healthcare

Whole Blood Bilirubin

The Chemical Pathology Department at National Women's Hospital, Auckland (a reference laboratory for neonatal bilirubin), was invited to evaluate the newly released whole blood neonatal bilirubin assay on the Radiometer ABL735 (Radiometer Pacific, Auckland). Two methods of comparison were used: the Hitachi 917 (Roche Diagnostics, Auckland) and the Photo B-H Meter V (NZ Medical and Scientific, Auckland).

The ABL735, using a minimum of 35µL, measures total bilirubin on the co-oximeter. The cells are lysed and the spectral curve scanned between 478 and 672 nm. As the spectrum of bilirubin can be distinguished from other components of the blood spectrum, it is possible to determine the concentration of bilirubin present. We found that there was a significant difference between our current methods and the ABL735 (greater than 35%). Radiometer has since released new software to correct this, and we hope to review this prior to the conference.

Comparison between Radiometer ABL735 and Chiron 865

The Accident and Emergency Department at Auckland Hospital had been considering the purchase of a whole blood analyser. Part of this process included a comparison of all analytes between the Radiometer ABL735 and the Chiron 865 (Bayer Diagnostics, Auckland).

Whole blood samples were processed initially on the Chiron 865 and then immediately after on the ABL735. The analyte comparisons, apart from glucose and lactate, were acceptable. Biases were similar to those evident in the external Quality Assurance Programme (RCPA-AACB) Blood Gases and Co-oximetry Programme, Cycle 24, July - December 1999. For glucose, the ABL735 measured on average 14% lower than the Chiron 865; for lactate, the ABL735 measured on average 20% lower than the Chiron 865. Comparisons with the Hitachi 917, using serum samples, are in progress.

Paracetamol (Acetaminophen) Interference

Patients in the Accident and Emergency Department are frequently administered paracetamol. We decided to investigate the possible interference of paracetamol on whole blood glucose and lactate. We found that glucose and lactate on the ABL735 is not affected by paracetamol, even at extremely toxic levels. Paracetamol even at normal therapeutic levels causes a slight increase in glucose (5-10%) on the Chiron 865. Lactate interference on the Chiron 865 was significant (greater than 20%) at normal therapeutic levels of paracetamol.

Features

Features of the ABL735 were that it appeared to be less prone to blockage from clots, required less maintenance, had facility for automated quality control sampling, displayed no interference from paracetamol, calibrated less frequently and contained on board video tutorials.

Features of the Chiron 865 were that it would attempt three calibrations before failing, has the facility to select individual analytes for both processing and calibration rather than all analytes and perhaps most importantly has manual sample control, which is especially useful for small volume samples.

A Family with Haemoglobin E and Beta Thalassaemia Condition

Shivani Ram, LabPlus, Auckland Healthcare

A Cambodian family living in New Zealand with Haemoglobin E/Beta thalassaemia disorder is presented. The father has Hb E disease and his wife has beta thalassaemia trait. Four of their five children have inherited the HbE/beta thal, but only one of them is severely affected, requiring regular transfusion and splenectomy. The father and the other three children with HbE/beta thal are clinically asymptomatic and are not transfusion dependant.

Beta globin abnormality with thalassaemia effect (beta thalassaemia and Hb E) are common in Asia. The Asian population in New Zealand has increased from 1.5% to 4.4% from 1986 to 1996. Haematology laboratories in New Zealand, therefore, will have an increased chance

of seeing such problems and should be aware of their laboratory diagnosis and clinical presentation. Furthermore, other genetic conditions, which can modify the clinical severity of these beta globin abnormalities, such as alpha thalassaemia, are also common in such populations.

This case illustrates the laboratory diagnostic features and complexity in antenatal molecular diagnosis of the beta globin problem.

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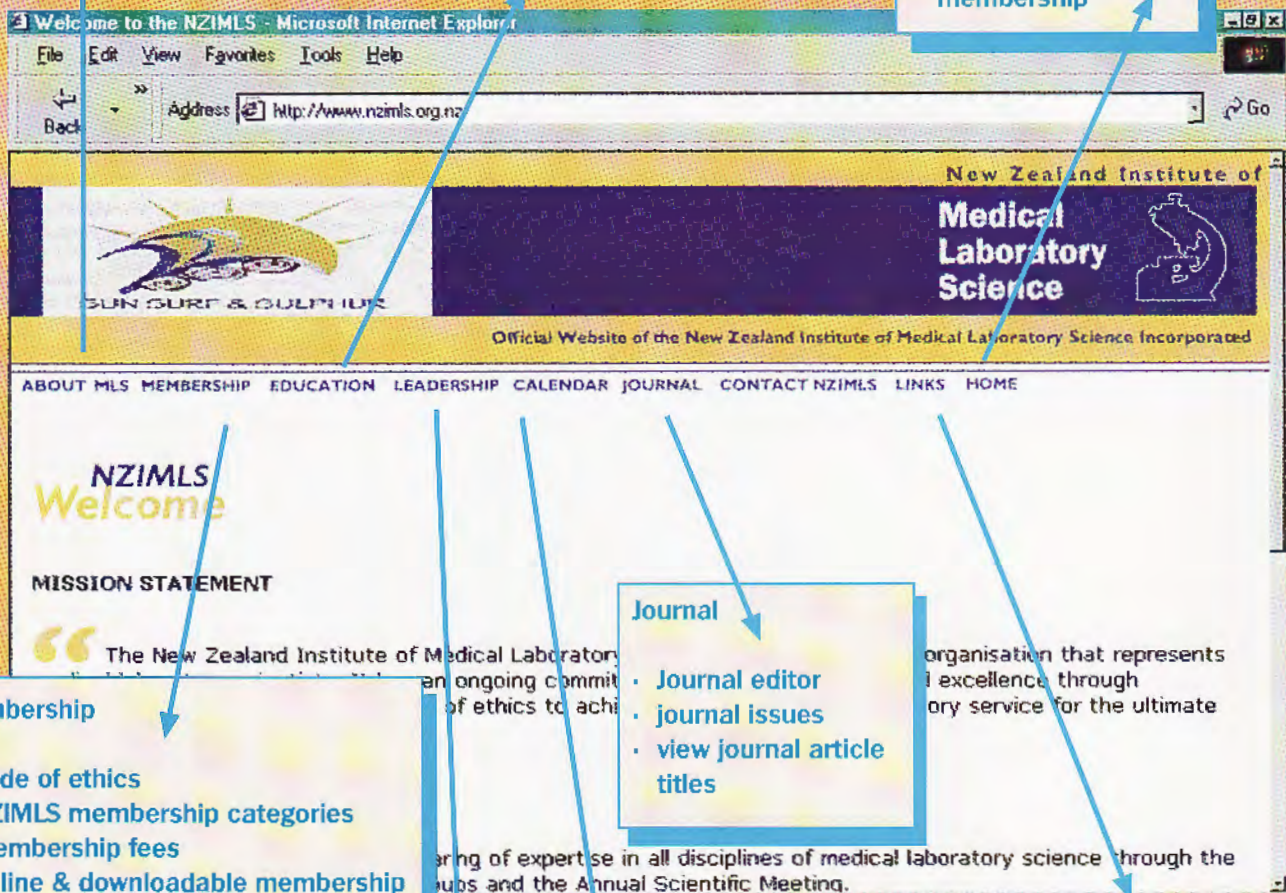
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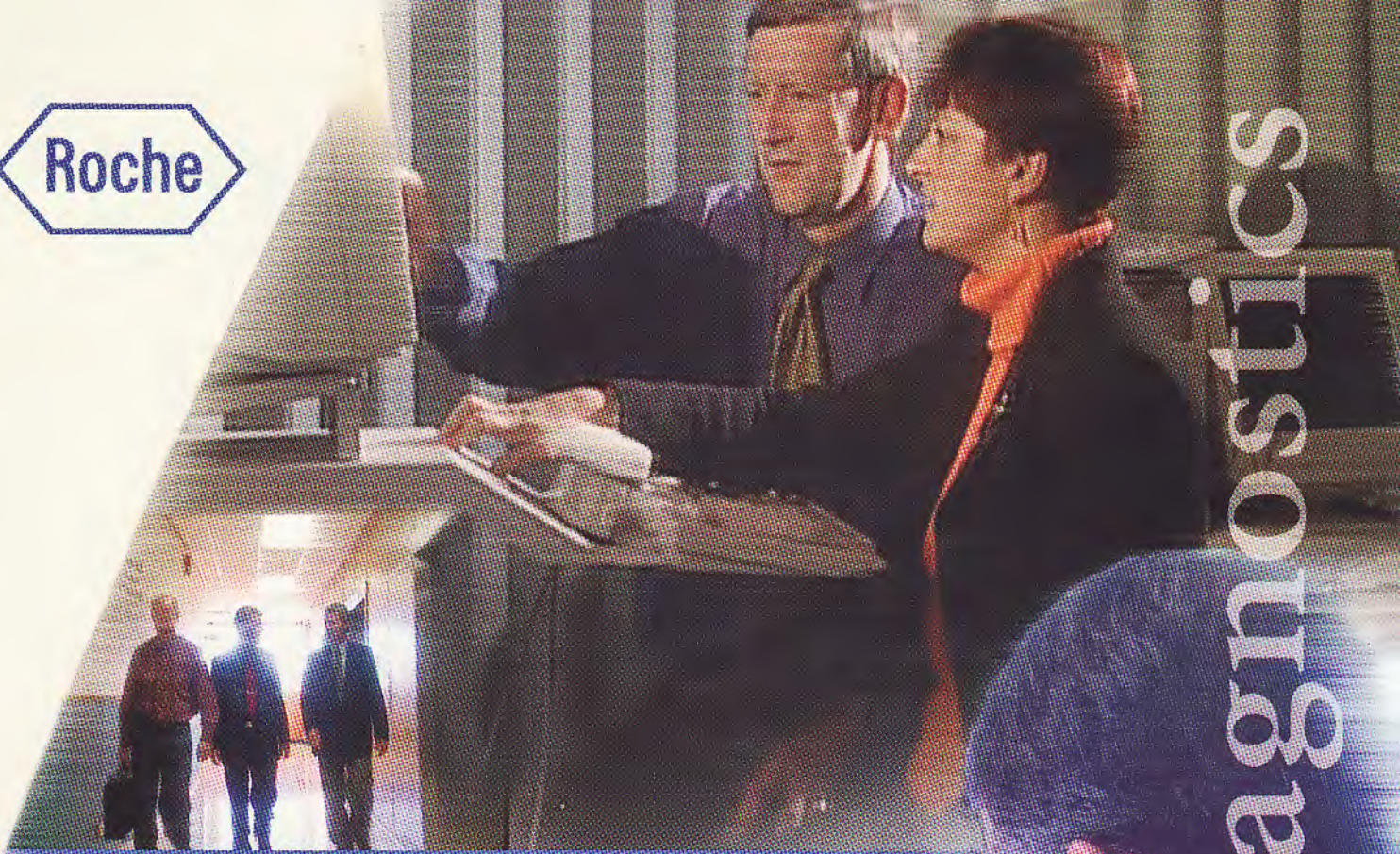
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