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* Tables should be typed on a separate page complete with a title at the top and footnotes at the bottom. The tables should be numbered as they appear in the text and must not contain vertical lines.
* Acknowledgements should be made to people and/or organisations who have made substantial contributions to the study. Authors are responsible for obtaining consent from those acknowledged. Financial contributions towards the study from granting bodies or commercial organisations must be stated.

Two copies of the manuscript are to be addressed to the Editor NZ J Med Lab Science, c/o Department of Medicine, Wellington School of Medicine, PO Box 7343, Wellington South, together with a letter from the corresponding author stating that the work is original, is not under consideration for publication elsewhere, and in the case of multi-authorship that all authors have contributed directly to the planning, execution, analysis or to the writing of the paper.
Alternative Site Testing: A Solution for the Isolated Country Hospital

Lorraine Craighead, Dr Martin Watts
Pathology Department, Balclutha Hospital

Introduction
Alternate Site Testing (AST), or Near Patient Testing may be defined as laboratory testing occurring outside the physical confines of the laboratory, but under the control of the central laboratory(1). Although AST has been well documented and researched in large hospitals, particularly in the USA and in the setting of critically ill patients(2) and in Intensive Care Units(3), there is no information in the literature concerning its use in small rural hospitals. We would like to discuss the successful introduction of AST in the setting of a small rural hospital in South Otago.

As is the case in many areas of New Zealand, the delivery of an appropriate laboratory service in Balclutha is related to the level of demand. There is a low population density and geographical remoteness. The rural and elderly population has a high level of service demand, and requires service provision within the district.

The hospital offers a specialist physician led service and includes the care of acute cardiac, respiratory and metabolic emergencies, demanding a level of laboratory service, which although it may be basic, must be prompt and accurate.

Geographically, Balclutha is a minimum of one hour by road from the nearest major laboratory services at Dunedin, however transport difficulties can produce clinically unacceptable delays in reporting of results from urgent cases, especially late at night.

The traditional small laboratory is very inefficient: Small volumes of samples are tested using instrumentation designed produce a large variety of analyses on many samples. Running costs are out of proportion to the number of analysis carried out and reagents are often wasted because they cannot be used quickly enough after reconstitution.

Not only are at least four staff members required to deliver a 24 hour service, but in order to keep those staff members gainfully employed during the day, the laboratory is often expanded to produce analyses such as LFT, lipids and iron studies which are not required on an urgent basis. This is a very wasteful use of scarce resources.

Introducing AST to the Hospital Setting
The laboratory in Balclutha is run by one full time equivalent who is responsible for quality control and maintenance of the instruments, on site analyses of urgent specimens during working hours, and most importantly, the training and supervision of the physicians who carry out the near patient testing. The laboratory also provides 24 hour a day troubleshooting backup service. The lab is an important source of information for other staff, and can often access help and advice from diverse sources within the organisation.

The introduction of AST to the three Medical Officers at Balclutha Hospital has been a gradual process. They provide the twenty-four hour medical cover, and are responsible for the initial assessment and management of all the acute medical problems. These doctors are responsible for requesting the out of hours laboratory testing, and also for the provision of most of the phlebotomy specimens. Doctors practice science, but are not laboratory scientists, and the training has to be organised accordingly. Doctors need to be able to deal with the technology with a minimum of thought so that they can focus all their attention on the patient. The laboratory has to work in close contact with the doctors, often at the patient's bedside. AST cannot be introduced successfully without co-operation from every other staff member.

Adequate time was allowed initially for discussion and thought on the subject, and protocols were prepared for familiarisation and training with the appropriate equipment.

All the documentation fulfilled the ISO9000 protocols for AST supplied by TELARC(4).

The choice of equipment has been a significant factor in the success of AST. Broadly speaking the machines used have been chosen as being user friendly, robust and reliable with a degree of portability and most importantly produce a hard copy printout of results. By the application of forward planning and the use of available guidelines(5), we have been able to proactively address potential problems of quality and accuracy of our testing procedures(6).

Each potential operator was introduced to the individual machines one at a time and adequate time was allowed for supervised use and repeated practice. When the user was deemed competent to use the equipment it was introduced into the clinical setting. The medical laboratory scientist has remained available 24 hours a day if help is needed. At all times meticulous documentation of training has been kept, and regular review of all users has followed. While the medical officers perform out of hours analyses, the responsibility for the accuracy and documentation of these analyses rests firmly with the laboratory. Results have been constantly reviewed by a knowledgeable laboratory scientist.

Practical Considerations and Cost Implications.
There are many practical aspects which need to be considered. As mentioned the choice of equipment is essential. The siting of equipment is important, accessibility must be appropriate for users without being in the way or in areas of too high use, as accidents inevitably happen in such areas.

Documentation of the results of AST is also of prime importance(7). There are important medico-legal considerations which must be addressed. Briefly this means that the results of all AST should be documented in the appropriate clinical and laboratory records for future reference with a note to the affect that these results have been obtained using AST. This has been facilitated where possible by the use of equipment which gives a hard copy printout of all results. We have found that where no hard copy exists the chain of information has been easily broken, with inadequate recording in some circumstances of the test done and the results obtained. This can be overcome but on the whole hard copy results are much preferred.

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Central laboratories are efficient because they spread fixed costs over a large number of units produced. Up to a point increases or decreases in volume have little effect on the cost of running the laboratory.

The cost structure for Near Patient Testing is quite different. The fixed costs while large, are not the largest components of the overall costs. Reagents make up a large portion of costs. When the volume increases, costs rise substantially, but when volume falls costs fall just as drastically. The substitution of variable costs for fixed costs has produced a very economic method of dealing with variable volumes.

The equipment remains at all times the responsibility of the laboratory. Regular maintenance, calibration and fault diagnosis and fixing should be carried out by trained personnel. Quality control of not only the equipment but also the users must be considered and at all times documented(8).

Discussion
We have identified several areas that we feel are the keys to the successful implementation of AST. The employment of motivated staff is essential. From the medical perspective adequate clinical experience is required. Laboratory results should always be interpreted in the light of the clinical picture, and this is even more important when the range of tests available is limited. The experience to decide which tests are essential, and the ability to interpret and act upon results is necessary. Appropriate training is vital, together with ongoing quality assurance. The provision of adequate back up in case of machine failure or other emergency is required. Good communication and feedback is needed, and all staff must be aware of the limitations as well as the advantages of AST.

Advantages of AST
AST has proved very effective at Balclutha Hospital. There have been several very positive advantages noticed by all concerned. Firstly important results can be accessed rapidly by clinicians, with minimal institutional delay. Secondly, and subjectively AST has improved clinical thinking by the simple expedient of the user concerned having to justify the time for doing each test, against the information gained. In the early hours of the morning, non relevant testing has been minimal. Thirdly communication between laboratory staff and clinicians has improved, with an appreciation on both sides of the other workload and problems. The financial aspects of AST are complex, and have been discussed previously(9,10).

Limitations of AST
There are distinct limitations to AST. Inevitably not all forms of laboratory testing are suitable, and some tests will be unavailable. For this reason adequate back up is required. Equipment may be non-portable or particular tests too complex. There is also the time consideration, as some tests may be too time consuming to fit in with the operator's other responsibilities. Finally the volume of testing has to be considered, if it becomes obvious that too much testing is being done out of hours then this can become a real burden on the staff performing the testing. This will lead to problems of resentment and disunity with serious consequences and probable discontinuance of AST.

The Future of AST
At the present time AST is in its infancy. The future increase in the use of AST is inevitable and will be driven by a number of factors. Chief amongst these will be the availability of newer equipment already on the market, which are small, robust, reliable and highly portable. These machines are able to perform multiple analyses on small specimens of whole blood. The range of test available will continue to grow and the capital cost of purchase and ongoing running costs will fall. Economic factors will also be looked at more closely in settings where the quantity of testing is low, and the maintenance of a full laboratory service is uneconomic.

Conclusions
Alternate Site Testing is not a quick fix solution to problems of budgeting or staffing. It will not work in all hospitals or in all clinical situations and this must be realised. The reasons for introducing AST must be appropriate and well thought out forward planning wholly essential. Some changes may be required in laboratory equipment. Changing of attitudes and mindsets amongst various professional groups may also be needed. These are more difficult to quantify and may provide a significant hurdle, as the nursing profession becomes multi-skilled maybe nurses will become involved with Alternate Site Testing.

However, in an appropriate setting and with the correct methods used AST can be introduced painlessly with benefits to all health and laboratory workers and the patients under their care.

Near patient testing in Balclutha is an extension of the parent laboratory. It is not an independent entity. It is tailored to meet the need for urgent tests in small volumes in geographically isolated areas. Those in large central laboratories may take comfort from the fact that nobody throws out their electric stove with the fan grill when they buy a microwave.

Technological change continues to simplify the operation of instruments, this will have an increasing impact on laboratories. Like it or not in the future labs will be smaller, with fewer technologists offering a more diverse mix of services to customers. As the expertise required to run the instruments of the past become redundant, flexibility and communication skills will become more important. We must think beyond the square. We may see the return of the multi-skilled laboratory scientist competent in the core disciplines of Chemical pathology, Hematology and Microbiology. Near Patient Testing may be only the first of many adaptations in the delivery of laboratory technology.

References
4. TELARC. Personal communication.

Greg Pennell, BMLS trainee (AIT), Haematology Department, Diagnostic Laboratory, Auckland.
Address for correspondence: Greg Pennell, Haematology Dept. Diagnostic Laboratory
PO Box 5728 Wellesley Street, Auckland.

Abstract
Neutrophil alkaline phosphatase is an enzyme which is involved in the breakdown of foreign materials and organisms within the body. The level of neutrophil alkaline phosphatase is consistently altered in a number of disease states and is therefore a useful diagnostic tool. Problems may unfortunately arise with the accuracy of the result when the assay is to be performed outside the collection area. This could be due to inappropriate and subsequent loss of enzymatic activity over time.

In order to investigate this problem several different protocols for specimen handling before analysis were evaluated. These included variations in fixation of the blood as well as different storage and transportation temperatures.

There were three different specimen types evaluated: fixed blood smears, unfixed blood smears, and whole heparinized blood. The temperatures investigated were: -20°C, 4°C, and room temperature. Prior to analysis the specimen moved through three temperature stages before analysis; storage prior to transportation, transportation, and storage after transportation. Temperature combinations were evaluated in regard to these stages.

From the analyses of variance performed on the data it was apparent that whilst the specimen type used had a significant impact on the results, the storage and transportation temperatures had no significance.

The method which was chosen, taking into consideration simplicity for the laboratory staff, was to make and fix the blood smears at the time of collection. The smears were then stored and transported at room temperature.

Keywords
Neutrophil Alkaline Phosphatase (NAP)

Introduction
Alkaline phosphatase is a hydrolytic enzyme that is capable of splitting phosphate esters. The enzyme is present in practically all tissue types within the body. It occurs in particularly high levels in intestinal epithelium, kidney tubules, osteoblasts, liver tissue, placental tissue and leukocytes (neutrophils). There are several different isoenzymes of alkaline phosphatase present within a population, although an individual rarely has more than two or three forms present. Generally alkaline phosphatase produced in one tissue varies subtly with the isoenzymes which have been produced in another tissue type. The major variation is in the heat stability of the enzyme, and there is also a slight difference in the electrical charge of the isoenzymes.

Neutrophils have the ability to phagocytose foreign organisms. To perform this the neutrophil needs an ability to store a wide variety of enzymes which can facilitate the breakdown of bacterial cell structures. These enzymes are contained within two distinct types of granules located within the cell's cytoplasm - azurophilic and specific granules. The enzyme alkaline phosphatase is located within the specific granules of the neutrophil. Alkaline phosphatase is identified by using the hydrolytic reaction of the enzyme coupled to a diazo salt binding reaction leading to a colour change. The neutrophil alkaline phosphatase (NAP) activity is measured using a scoring system devised by Kaplow.

Neutrophil alkaline phosphatase (NAP) activity alone is not diagnostic of any particular disease state, it is however consistently altered in a number of disease states. This laboratory test can then be utilised along with other clinical data to distinguish between two apparently similar disease states.

Low levels of NAP activity are repeatably found in a number of conditions - Philadelphia (Ph) positive Chronic Myeloid Leukemia (CML), Hypophosphatasia, Paroxysmal Nocturnal Haemoglobinuria (PNH), and occasionally Acute Myeloid Leukemia (AML). The occurrence of high NAP levels are recognised in a larger number of, and more common disease states - Aplastic anaemia, Polycythaemia Rubra Vera, Leukemoid reactions, Myeloid metaplasia, Infection and Stress conditions. During pregnancy the NAP activity rises over the course of gestation reaching a peak when the mother reaches full term. Although high NAP activity is recorded in patients with recurrent infections it has been widely observed that limited NAP activity is recorded in patients with recurrent infections. The recurrent infections that these patients experience is usually due to the absence of the specific granules (which contain the alkaline phosphatase) in the patients' neutrophils rather than dysfunctional enzymes.

As mentioned previously the stability of alkaline phosphatase at increased temperatures is one way to distinguish the different isoenzymes. This differentiation is done at relatively high temperatures (50 - 60°C), regarding this investigation it is the storage temperature which is being altered (-20°C, 4°C, R) so high temperature denaturation isn't an issue.

A decrease in temperature below body temperature decreases the reaction rate. However, an increase in temperature over certain levels can denature the enzyme and this causes the enzyme to be inactive by destroying its structure; this also decreases the reaction rate. Lowering the temperature preserves the integrity of the enzyme. It is able to continue working when the temperature increases to a level that is able to sustain the enzymes' activity. The situation with neutrophil alkaline phosphatase is slightly more complicated than this. As the NAP activity must be visualised in the neutrophil, the integrity of the neutrophil must be maintained. Difficulty arises with this because the medium that we are dealing with is blood. Components within the blood, such as the macrophages, are designed to remove dead and degraded material from within the body and blood.
For each cell to maintain its integrity it must utilise energy (glucose and ATP). Unfortunately the small sample of blood that is collected for analysis contains only a limited source of energy. When this energy source is exhausted the cells start to degrade. So whilst the alkaline phosphatase enzyme itself may be intact and functional the cell in which it is contained may have been destroyed. Therefore we must slow down the rate of energy expenditure by the cells in the blood sample. This can be controlled by temperature. If we decrease the blood samples’ temperature then the cellular activity decreases and so does the rate of neutrophil degradation.

At present in our laboratory all specimens to be tested for neutrophil alkaline phosphatase within the region are brought to a central testing laboratory. Several blood smears are made, fixed appropriately and then frozen at -20°C until analysed.

Specimens from other regions are collected and smears made and fixed at the collection centre (for approximately 5-8 hours) at -20°C before being transported at approximately 4°C to the central laboratory for testing. They are then frozen at -20°C on arrival.

The aim of this experiment was to determine if the current method, as described above, is the most appropriate for the situation, taking into consideration storage and transportation temperature between the centres.

Other protocols under evaluation are combining different storage and transportation temperatures (-20°C, 4°C, R.), and alternative fixation times within the process.

Materials and Methods

Samples were collected into 4 ml Heparin vacutainer collection tubes (Greiner Labortechnik, Germany). There were a number of temperature combinations evaluated for each of the three sample types; whole blood, fixed blood smears, and unfixed blood smears. These temperature combinations are outlined in table 1.

<table>
<thead>
<tr>
<th>1st Storage Temp. (°C)</th>
<th>Transportation Temp. (°C)</th>
<th>2nd Storage Temp. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-20</td>
<td>4</td>
<td>Rt</td>
</tr>
<tr>
<td>-20</td>
<td>4</td>
<td>Rt</td>
</tr>
<tr>
<td>4</td>
<td>Rt</td>
<td>-20</td>
</tr>
<tr>
<td>4</td>
<td>Rt</td>
<td>-20</td>
</tr>
<tr>
<td>Rt</td>
<td>4</td>
<td>-20</td>
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<tr>
<td>Rt</td>
<td>4</td>
<td>-20</td>
</tr>
<tr>
<td>-20</td>
<td>4</td>
<td>Rt</td>
</tr>
<tr>
<td>4</td>
<td>Rt</td>
<td>-20</td>
</tr>
<tr>
<td>4</td>
<td>Rt</td>
<td>-20</td>
</tr>
<tr>
<td>Rt</td>
<td>4</td>
<td>-20</td>
</tr>
</tbody>
</table>

To minimise complications the procedure was performed at three separate times using the same type as the distinguishing factor. In the case where whole blood was used the sample tube was not frozen (at -20°C) and those options have not been evaluated. The blood smears were made immediately after collection using Esco frosted slides (Biolab, NZ). An exception was the whole blood sample; the blood smears were made and fixed after transportation. The fixed smears were then stored at the appropriate temperature.

At the time of collection an NAP score was determined on the sample. This was used as a baseline with which to determine any change in NAP activity. This change results from the effect that the storage temperatures have on the sample.

The time allowed for specimen storage before and after transportation was eight hours. The transportation time was set at five hours. Changes in temperature for the storage and transportation of the sample were simulated by leaving the specimens on the bench (R.), in the fridge (4°C), or in the freezer (-20°C) for the appropriate period of time.

The staining technique for the NAP utilises the hydrolysis of Naphthol AS Phosphate. This liberates the naphthol which binds to Fast Blue BBN producing a blue precipitate at the reaction site. Once the blood smears had been stained they were lacquered (to protect the smear) with acrylic lacquer (Spraystore CPC-1000). After lacquering the NAP score was determined using a light microscope (Olympus BH2). In addition to the NAP score the specimens were evaluated with a number of criteria to determine the quality of the material available for analysis. The four quality criteria included were; the appearance and intactness of the neutrophils, the definition of the stain within the neutrophils, the distinction of the stain and the presence of any background contamination. These criteria were each given a value ranging from one to seven; the lowest number represents the poorest quality, and up to seven relating to a high standard of quality for that particular criteria.

The data was then subjected to an analysis of variance (ANOVA) to determine which, if any, of the factors was significant to the outcome (NAP score and quality of the blood smear).

Results

There were seventy-two different combinations which were analysed and compared to one another.

The data which was collected is represented graphically (graphs 1-5) based on the specimen type used, fixed, unfixed, or whole heparinized blood. The graphs show changes in the five responses as the specimen type is altered.

With this data five separate one-way analyses of variance (ANOVAs) were performed using Minitab version 10.5. The information then available determines whether there is a significant difference over the specimen types. Five ANOVAs were calculated to determine if any detectable significance was found over all of the responses or if it was limited to specific key points. The five analyses are shown in table 2. A p value of < 0.05 was deemed statistically significant.

As a result of the information obtained a balanced ANOVA was performed on the data from the fixed specimen. This will determine any significance for the storage and transportation temperatures if the specimen is fixed at the time of collection. The balanced ANOVA is shown in table 3.

Discussion

After evaluating the results from the one-way analysis of variance for the specimen type it was apparent that this variable had a significant impact on all of the five responses. This can be concluded from evaluating the 'p' value (level of significance) for each of the calculations. This level of significance is arbitrarily set at 5% (0.05). If the 'p' value is less than 0.05 the sample populations concerned are greater than 95% different - there is a significant difference.
Table 2. Summary of the five ANOVAs for each of the separate quality criteria to determine any significance of specimen type.

<table>
<thead>
<tr>
<th>Quality Criteria</th>
<th>Specimen Type</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>95% Confidence Interval</th>
<th>'p' value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAP Difference</td>
<td>Fixed</td>
<td>10.74</td>
<td>8.71</td>
<td>2.030 - 19.45</td>
<td>0.000</td>
</tr>
<tr>
<td>Appearance</td>
<td>Fixed</td>
<td>6.074</td>
<td>0.829</td>
<td>5.245 - 6.903</td>
<td>0.000</td>
</tr>
<tr>
<td>Uniformity</td>
<td>Fixed</td>
<td>6.448</td>
<td>0.580</td>
<td>5.901 - 7.061</td>
<td>0.000</td>
</tr>
<tr>
<td>Background</td>
<td>Fixed</td>
<td>6.185</td>
<td>0.622</td>
<td>5.563 - 6.807</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Unfixed</td>
<td>3.407</td>
<td>1.309</td>
<td>2.098 - 4.716</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Whole</td>
<td>3.389</td>
<td>1.195</td>
<td>2.194 - 4.584</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unfixed</td>
<td>3.407</td>
<td>1.907</td>
<td>1.500 - 5.314</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Whole</td>
<td>3.167</td>
<td>0.985</td>
<td>2.182 - 4.152</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. 'P' values from balanced ANOVA for fixed specimens.

<table>
<thead>
<tr>
<th>1st Storage Temp</th>
<th>Transport. Temp</th>
<th>2nd Storage Temp</th>
<th>'p' value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAP Difference</td>
<td>0.089</td>
<td>0.811</td>
<td>0.432</td>
</tr>
<tr>
<td>Appearance</td>
<td>0.407</td>
<td>0.711</td>
<td>0.536</td>
</tr>
<tr>
<td>Definition</td>
<td>0.609</td>
<td>0.845</td>
<td>0.157</td>
</tr>
<tr>
<td>Uniformity</td>
<td>0.477</td>
<td>0.477</td>
<td>0.263</td>
</tr>
<tr>
<td>Background</td>
<td>0.545</td>
<td>0.704</td>
<td>0.333</td>
</tr>
</tbody>
</table>

Once it has been established that the specimen type does have a significant impact on the results it must be determined which is significantly better, fixed blood smears, unfixed blood smears, or whole heparinized blood. The most important response and therefore best indicator of this is how much the NAP score has changed from the baseline score which was determined at the time of collection. The response that is dealt with is the NAP difference. The ideal, is of course, for the NAP score to change very little, with a low mean and a small standard deviation. The mean and standard deviation for fixed smears is 10.74 and 8.71 respectively, these are both much lower than for the unfixed blood smears and whole blood (33.41 / 33.04 and 73.94 / 12.00 respectively). Fixing the blood smears at the time of collection is therefore the preferable method and one by which further calculations are determined.

Possible reasons for the degradation of the other two specimen types are:

- Changes in the temperature which can cause condensation to form on the surface of the slide. The cells are then in a hypotonic solution which can lead to cell lysis resulting in an increase in background contamination.

- Because the cells are still 'alive' they are respiring, utilising ATP and degrading. This would invariably lead to a decrease in NAP activity.

- The cells haven't degraded as much with the whole blood because there isn't the same condensation formation occurring. This is why there isn't significant difference with the background contamination between the fixed blood smears and the whole blood.

Once the best specimen type to use has been determined it was necessary to evaluate any significance with the storage and transportation temperatures. This is calculated using only the data derived from the fixed blood smears. A balanced ANOVA was carried out on the data to evaluate any significance.

Upon calculating the 'p' values obtained from this ANOVA we find that none of the 'p' values fall within the 5% (0.05) significance threshold. Therefore we can conclude that once the specimen has been initially processed there is no significance as to whether the specimens are stored or transported at either -20°C, 4°C, or at room temperature.

The method used for the specimen storage and transportation should, while taking into consideration the information which has resulted from this experiment, also provide simplicity for the laboratory staff and be the most economically sensible method.

**Conclusions**

The type of specimen used has significant impact on the result. The best specimen to use are blood smears which have been fixed as soon after collection as possible. Once the blood smear has been fixed, the storage and transportation temperatures have no significant influence on the outcome. The most appropriate method to use is to make and fix the blood smears at the time of collection. Once that is completed the smears can be stored and transported at room temperature.

**Recommendations**

1. Make blood smears from heparinized blood and fix as soon as possible after collection.

2. If the specimen requires transportation it can be transported and stored prior to transportation at room temperature.

3. When the specimen (fixed blood smears) arrives at the laboratory for testing it can be stored at room temperature.

**Acknowledgments**

I would like to acknowledge Diagnostic Laboratory Auckland as well as several Haematology staff members, especially Ross Anderson, Karen Young and Angela Mason for their assistance during the course of this study. I would also like to thank a number of lecturers at the Auckland Institute of Technology, in particular Dr. Charles Small for supervising the research.

**References**


Figure 4. Uniformity.

Figure 5. Background.

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An Evaluation of the 'Mouse Model' in the Study of the Virulence of Coagulase-Negative Staphylococci Isolated from Sheep Subclinical Mastitis.

Angeliki R Burriel DVM, MSc, PhD, MRCVS, Royal Veterinary College, Department of Farm Animal and Equine Medicine and Surgery, Boltons Park, Potters Bar, Herts, EN6 1NB, United Kingdom.
Address for correspondence: Dr A Burriel, PSC 1, Box 1878, APO AE 09009-1800, U.S.A.

Abstract

The mouse model in the study of the virulence of coagulase-negative staphylococci was evaluated by inoculation of mice via the intraperitoneal and intramammary routes. One hundred fifty six adult mice, and 401 12-14 day old were inoculated intraperitoneally. Additionally, 75 lactating mice were inoculated under the teat base. A total of 45 coagulase-negative staphylococci and 5 coagulase-positive were used as inoculum. From the mice inoculated intraperitoneally, only four adults and 55 young mice died within 48 hours from inoculation. Of the remaining mice, 87 adults and 138 young showed clinical and post-mortem signs of illness. The mean spleen weight of 115 intraperitoneally inoculated mice indicated some weight differences possibly associated to the virulence of staphylococci. Fifteen of these mammary glands exhibited histopathological lesions that varied from purulent inflammation caused by the micro-organisms. Fifty of these mammary glands had been isolated from clinical mastitis of sheep. Coagulase-negative staphylococci associated with subclinical mastitis caused the severest lesions were the ones isolated from clinical mastitis of sheep. Coagulase-negative staphylococci associated with subclinical mastitis caused only very mild changes that varied from mouse to mouse and strain. Pleiotropy of signs was high in both routes and ages of mice making this model impractical and expensive in the study of a large number of staphylococcal strains isolated from sheep subclinical mastitis. However, the model seems to be useful in the differentiation of very virulent strains causing clinical mastitis from a virulent or of reduced virulence strains isolated from sheep mammary glands. The most virulent strains were identified as Staphylococcus aureus and Staph. intermedius which had been isolated from clinical mastitis of sheep and Staph. simulans, Staph. warneri, Staph. epidermidis and Staph. haemolyticus which had been implicated in subclinical mastitis of sheep. The species of coagulase-negative staphylococci found as the most virulent in the mouse model are those mostly frequently isolated from sheep subclinical mastitis.

Key words

Coagulase-negative staphylococci, subclinical mastitis, mice, laboratory animals, experimental study.

Introduction

Mice have been used extensively as experimental models in the study of bacterial pathogenicity. In this context, the mouse model has been used, with varying success, to study the pathogenicity of Staph. aureus and its products (1, 2), but it has been rarely used in the study of coagulase-negative staphylococci (C-NS). The model has been found to be influenced by the method of administration, resistance of mice and size of the inoculum (3). It is suggested that for successful experimental results various forms of intervention should be used; inoculations should be with more than one route (4), resistance should be overcome with the use of immunocompromised mice (5) or use of a foreign body (6, 7) and the inoculum should contain between 10⁴ - 10⁷ colony forming units per ml (8).

The mouse mastitis model was developed (9) to study the virulence of Staph. aureus strains isolated from bovine mastitis. A simplified version of this model (10) was used to compare the invasiveness of Staph. aureus and Staph. epidermidis. The mouse mammary gland has been used to study pathological lesions produced by various strains of staphylococci isolated from cases of mastitis (11, 12), the effect of mutants that lack coagulase or α-haemolysin (13) or the ability of staphylococci to adhere in vivo to mammary epithelial cells (14).

In light of the information available, the objective of the present experiment was to study the pathogenicity of various strains of C-NS inoculated into young and adult mice via the intraperitoneal or intramammary route.

Materials and Methods

Mice and inoculum: A total of 644 mice strain CRH were used for this experiment and were supplied by Glaxo-Wellcome, Ltd, U.K. They were 243 adult female mice of which 75 gave birth to 401 young mice used in the present experiment. Mice were kept in separate cages, except lactating mice that were kept with their offspring.

The strains of staphylococci used at various experiments totalled 45 C-NS and five coagulase-positive (CP) (Table 2). Most C-NS strains, with the exception of one strain (N50), had been isolated from subclinical mastitis (SCM) of sheep. The five CP strains and strain N50 had been isolated from cases of clinical mastitis of sheep. The strains were cultured in either 110 Staphylococcus selective medium (110 SM) (Difco, USA), or Todd-Hewitt (TH) broth (Unipath, UK) at 37°C for 24 hours. The cultures were centrifuged at 1500 g and 4°C for 20 minutes. The bacteria were resuspended in sterile phosphate buffered saline (PBS) to a density of approximately 10⁷ colony forming units (cfu) per ml before inoculation into mice. In addition to mice inoculated with live organisms, 96 young mice were inoculated with sterile supernatant of 11 strains of C-NS and five CP. The supernatant was filtered through a low protein binding filter with a pore size of 0.45 μ (Millipore, UK). All staphylococcal strains had been assigned to species by the Staph-Zym Kit (Rosco Laboratories, Denmark).

Intraperitoneal inoculation procedure: Adult mice: One hundred fifty six adult mice were inoculated by the intraperitoneal route. One ml of inoculum was inoculated with a 26 gauge needle in the right flank. Four mice were inoculated with one or other of 38 strains of C-NS and one CP strain used as a positive control (Table 1). Twelve mice were inoculated with only one ml of sterile PBS and served as negative controls.

Young mice: Two hundred fifty six adult mice 12-14 days old were inoculated with half the adult dose of inoculum in the right flank. Five mice were inoculated with one or other of 45 strains of C-NS, most of which had also been inoculated into adults, and five CP strains (Table 2). A group of 45 mice received only 0.5 ml of sterile PBS. In addition to live organisms, 96 young mice were inoculated intraperitoneally with 0.5 ml of the sterile supernatant of 11 strains of C-NS and five CP cultured in TH. Ten mice were inoculated with the same amount of only sterile TH.
Intramammary inoculation procedure:
Twenty four strains of C-NS determined by the intraperitoneal inoculations in adult and young mice as the most virulent, and one CP (Table 3) were inoculated into the teat base of 75 lactating mice. The mice were in their 12th to 14th day of lactation. The inoculations were performed under general anaesthesia induced in a chamber containing ether vapour. The R4 mammary gland was inoculated with live micro-organisms using a hypodermic needle and the L4 mammary gland with sterile PBS. The teat was flexed gently with forceps and approximately 150 μl of inoculum containing approximately 10^7 bacteria per ml was deposited under the teat base and into the area of the teat cistern. The mothers were separated from their inoculated young mice for only one hour after inoculation.

Clinical observations and laboratory procedures

a. Intraperitoneal route: The inoculated mice were observed for clinical signs of disease 12 and 24 hours after inoculation and every 24 hours thereafter. Two thirds of the inoculated adult mice were euthanatized by cervical dislocation 48 hours after inoculation and the remaining one week. The abdomen and thorax were thoroughly cleaned with 70% clinical alcohol and dissected as to expose the internal organs. All the adults were examined post-mortem for pathological signs of their internal organs related to staphylococcal infection. A few drops of blood collected directly from the heart was cultured in Brain Heart Infusion broth (BHIB) (Unipath, UK) to detect the presence of bacteraemia. The surfaces of the liver and spleen were seared with the hot tip of a scalpel blade and incised with a sterile blade. A small amount of tissue parenchyma was removed and cultured in BHIB at 37°C for 24 hours. The isolated staphylococci were assigned to species level using the Staph-Yzm kit. Only if the species was that of the inoculum the sample was determined positive. Staphylococcal virulence among young mice was determined by clinical observations and mortality rates. Additionally, 125 (115 infected and 10 controls) young mice were euthanatized and their spleens were collected and weighed at 48 hours post-inoculation.

b. Intramammary route: The inoculated glands were palpated every 24 hours for up to one week for evidence of inflammation at the R4 gland area. Fifty mice were euthanatized 48 hours after inoculation and the remaining one week. Euthanasia was performed with intraperitoneal injection of euthatal (RMB Animal Health Limited, UK). After thoroughly swabbing the abdomen with 70% clinical alcohol, the mammary glands (inoculated and control) were exposed and incised with a sterile scalpel blade. A small piece of tissue was collected and cultured in BHIB at 37°C for 24 hours. The remaining gland was fixed in 25 ml of 10% buffered formalin, embedded in wax, cut and stained with Haematoxylin-Eosin stain (Glaxo-Wellcome, UK). Samples were examined for histopathological changes with light microscopy.

Results

Intraperitoneal route: Adult mice: The clinical signs observed among the inoculated mice were used to define mice as ill if they exhibited signs of rough coat, hunched back and shivering or difficulty in breathing, lack of eating and drinking and lack of movement. Of the 156 inoculated mice four died within 48 hours after inoculation and 87 showed clinical or post-mortem pathological changes (Table 1). Staphylococci were isolated from various organs and lesions of 26 mice, but from the blood of only the four dead mice. Three of the dead mice had been inoculated with a CP strain.

Young mice: Clinical illness was determined as with the adults and mortality rate. Post-mortem examination was not performed with the young mice. From the 225 mice inoculated with live C-NS, 30 died within one week from inoculation. Of those, 25 mice had been inco-

Table 1. Species and number of staphylococci inoculated into adult mice, most virulent species, and clinical or post-mortem signs of illness.

<table>
<thead>
<tr>
<th>Species of Staphylococci</th>
<th>Number of strains</th>
<th>Virulent strains</th>
<th>Clinical observation</th>
<th>Proportion of affected mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>aureus</td>
<td>1</td>
<td>1</td>
<td>Large</td>
<td>28.8</td>
</tr>
<tr>
<td>capitis</td>
<td>4</td>
<td>2</td>
<td>Spleen</td>
<td>3.8</td>
</tr>
<tr>
<td>epidermidis</td>
<td>5</td>
<td>2</td>
<td>Abscesses</td>
<td>18</td>
</tr>
<tr>
<td>haemolyticus</td>
<td>1</td>
<td>1</td>
<td>Bacteria (+) organs or abscesses</td>
<td>16.7</td>
</tr>
<tr>
<td>lentus</td>
<td>1</td>
<td>0</td>
<td>Hyp-Cong IO*</td>
<td>4</td>
</tr>
<tr>
<td>scirri</td>
<td>1</td>
<td>0</td>
<td>Dead</td>
<td>2.6</td>
</tr>
<tr>
<td>shleiferi</td>
<td>2</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>simulans</td>
<td>8</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>xylosus</td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>warneri</td>
<td>10</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>species</td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>39</td>
<td>17</td>
<td></td>
<td>58.3</td>
</tr>
</tbody>
</table>

* hyperaemia or congestion of internal organs.

Table 2. Species and number of staphylococci inoculated into young mice, most virulent species, and clinical signs of illness.

<table>
<thead>
<tr>
<th>Species of Staphylococci</th>
<th>Number of strains</th>
<th>Virulent strains</th>
<th>Clinical observation</th>
<th>Proportion of affected mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>aureus</td>
<td>3</td>
<td>3</td>
<td>Ill receiving live staphylococci</td>
<td>55.2</td>
</tr>
<tr>
<td>capitis</td>
<td>5</td>
<td>1</td>
<td>Dead receiving</td>
<td>22</td>
</tr>
<tr>
<td>epidermidis</td>
<td>5</td>
<td>2</td>
<td>Live staphylococci</td>
<td>15.6</td>
</tr>
<tr>
<td>haemolyticus</td>
<td>1</td>
<td>0</td>
<td>Ill receiving filtrates</td>
<td>19</td>
</tr>
<tr>
<td>intermedius</td>
<td>2</td>
<td>2</td>
<td>Dead receiving</td>
<td></td>
</tr>
<tr>
<td>lentus</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>scirri</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>shleiferi</td>
<td>2</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>simulans</td>
<td>8</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>xylosus</td>
<td>5</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>warneri</td>
<td>10</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>species</td>
<td>7</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>50</td>
<td>23</td>
<td></td>
<td>58.3</td>
</tr>
</tbody>
</table>
Spleen weight of young mice: Seventeen strains of staphylococci were examined for their influence on the spleen of young mice. The mean weight of spleens from the mice inoculated with nine of the live strains was double or more than that of the control mice (Table 3). The mean spleen weight of mice inoculated with live organisms and those inoculated with filtrate from the same strain differed in only two of the five strains examined (Table 3). The mean weight of spleens from mice inoculated with live micro-organisms or their filtrate was similar with the exception of strain N108 (Table 4). Three strains (N28a, N56h, N157a) had been re-isolated from adult mice inoculated with strains N28, N56 and N157, and were used as inoculum for young mice.

Table 3. Mean weight of spleen from 12-14 day old mice inoculate with live C-NS or their filtrate.

<table>
<thead>
<tr>
<th>Bacterial Strain</th>
<th>mean weight (g)</th>
<th>Bacterial Strain</th>
<th>mean weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N34*</td>
<td>0.20</td>
<td>N106</td>
<td>0.08</td>
</tr>
<tr>
<td>N47a</td>
<td>0.11</td>
<td>N108</td>
<td>0.12</td>
</tr>
<tr>
<td>N47h</td>
<td>0.09</td>
<td>N108h</td>
<td>0.06</td>
</tr>
<tr>
<td>N28</td>
<td>0.12</td>
<td>N131</td>
<td>0.16</td>
</tr>
<tr>
<td>N28a</td>
<td>0.11</td>
<td>N133</td>
<td>0.05</td>
</tr>
<tr>
<td>N46</td>
<td>0.05</td>
<td>N142</td>
<td>0.09</td>
</tr>
<tr>
<td>N46h</td>
<td>0.05</td>
<td>N145</td>
<td>0.07</td>
</tr>
<tr>
<td>N50</td>
<td>0.06</td>
<td>N145h</td>
<td>0.07</td>
</tr>
<tr>
<td>N50h</td>
<td>0.04</td>
<td>N157</td>
<td>0.04</td>
</tr>
<tr>
<td>N56</td>
<td>0.06</td>
<td>N157a</td>
<td>0.04</td>
</tr>
<tr>
<td>N56h</td>
<td>0.05</td>
<td>N158</td>
<td>0.09</td>
</tr>
<tr>
<td>negative control</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*N CP strains. *mice inoculated with sterile filtrate.

Intramammary route: Twenty four hours after inoculation, all strains had one or more mice with an affected gland. Fourteen of the 75 glands inoculated showed no evidence of any clinical changes, 13 showed only light localised hardness at the site of inoculation and the remaining 48 showed mammary gland enlargement and hardness (Table 4). Only four of the 25 mice kept for one week exhibited hardness close to the inoculation site at last examination and all had a teat base abscess from which the species of the inoculum was isolated.

Table 4. Species and number of staphylococci inoculated into lactating mice, most virulent species, and clinical signs of observations.

<table>
<thead>
<tr>
<th>Species of Staphylococci</th>
<th>Number of strains</th>
<th>Virulent strains</th>
<th>Clinical observation</th>
<th>Proportion of affected mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>aureus</td>
<td>1</td>
<td>1</td>
<td>Bacteria (+)</td>
<td>17.3</td>
</tr>
<tr>
<td>capitis</td>
<td>1</td>
<td>0</td>
<td>Abscesses</td>
<td>30.7</td>
</tr>
<tr>
<td>epidermidis</td>
<td>2</td>
<td>2</td>
<td>Large gland</td>
<td>2.7</td>
</tr>
<tr>
<td>haemolyticus</td>
<td>1</td>
<td>1</td>
<td>Small gland</td>
<td>9.3</td>
</tr>
<tr>
<td>simulans</td>
<td>8</td>
<td>2</td>
<td>White gland</td>
<td>13.3</td>
</tr>
<tr>
<td>xylosus</td>
<td>1</td>
<td>1</td>
<td>Hypersemia</td>
<td>10.7</td>
</tr>
<tr>
<td>warreni</td>
<td>9</td>
<td>3</td>
<td>Oedema</td>
<td>13.3</td>
</tr>
<tr>
<td>species</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>25</td>
<td>11</td>
<td></td>
<td>70.3</td>
</tr>
</tbody>
</table>

Histological examination of 46 of the control glands did not show any signs of abnormality. However, ten control glands had a small increase in monocytes and the remaining showed some signs of involution. The last were all from mice euthanized at one week post-inoculation. Of the 61 inoculated glands that showed some clinical signs, 11 did not have any evidence of histopathological changes and 50 had some histopathological lesions. The histopathological lesions varied among the strains and inoculated mice. The glands of three mice inoculated with one CP strain, one mouse each inoculated with one or other of two C-NS, one of which was a capsule possessing strain (N50) exhibited acute inflammation involving infiltration of the interalveolar connective tissue with inflammatory cells and some purulent exudate in mammary alveoli (Fig. 1). Five other glands showed mild evidence of mixed acute and chronic lesions exhibiting light neutrophilic and monocytic infiltration and light vacuolation or an increase in nucleus to cytoplasm ratio of epithelial cells, an indication of epithelial cell involution. Most of the inflammatory cells were present in the interalveolar spaces. The 41 remaining affected glands showed mainly changes of a chronic nature that ranged from accumulation of monocytes in connective tissue and around involuted alveoli (Fig. 2) to glandular involution and increased interalveolar connective tissue, with areas of complete involution of glandular epithelia.

C-NS strains that caused more than two signs of clinical, post-mortem or histopathological illness in the various experiments, were considered as virulent. Only sixteen strains inoculated intraperitoneally into adult mice, 18 inoculated into young mice and 10 inoculated under the teat base were considered as the most virulent (Tables 1, 2 & 3). From the 24 strains that were inoculated into mice with all three routes, only five were included among the most virulent in all three routes. They were four identified as Staph. warneri (N50, N93, N141, N142) and one identified as Staph. simulans (N56). From the 38 inoculated intraperitoneally into both adult and young mice only nine were virulent in both ages.

Discussion and Conclusion

The 1105M medium was used to increase the production of slime (15), which has been found to increase the virulence of Staph. epidermidis for mice (8). This medium was found in preliminary tests to be toxic to young mice when filtrate was inoculated intraperitoneally, thus TH was used to culture the organisms for the production of filtrates.

Differences in the virulence of staphylococci observed previously with different routes of administration (4) were confirmed here. The one CP strain inoculated intraperitoneally and under the teat base was virulent in all routes, but caused death only after intraperitoneal inoculation, suggesting that death of mice should not be considered as the most important indicator of staphylococcal virulence. The C-NS were not causing any deaths to adult mice regardless of route, either because they are of low virulence or adults are resistant to these organisms. Resistance encountered among adult mice could have resulted from the sex of the mice. All inoculated adults were females that are thought to be more resistant than males (16). Resistance to inoculated C-NS among young mice was high and could have resulted from their age. Some (7, 17) suggest that young mice become resistant to staphylococcal infection at about seven to eight days of age. The age of 12-14 days was considered here as more appropriate for confronting a more mature immune system that could be an important factor in staphylococcal infections of lactating ovine mammary glands. In addition, previous work (11) had suggested that this age was appropriate for inoculation with C-NS isolated from ovine subclinical mastitis. The very low mortality rate among young mice as
Figure 1. Mouse mammary tissue exhibiting acute inflammation. Large number of neutrophils is observed in the alveoli. The gland was inoculated with Staph. haemolyticus strain N148. x100

Figure 2. Mouse mammary tissue exhibiting chronic inflammation. Monocytes are infiltrating the subepithelial tissue. The gland was inoculated with a Staph. simulans, strain N46 and examined at one week post-inoculation. x400

compared to that reported by others (11) could have resulted from the size of the inoculum. Use of an inoculum as large as that of the adults (11) was considered as disproportional to the body weight of a young mouse and to its degree of immune system maturity.

The post-mortem examination of euthanized mice indicated that infection from C-NS could cause enlargement of the spleen, isolation of C-NS from internal organs and abscesses of the parenchymal organs and peritoneum. Similar lesions were observed previously (16) and have been suggested as possible markers of virulence among strains of C-NS. Splenomegaly and internal organ abscesses were a frequent finding and should be investigated further as a measure of staphylococcal virulence. Splenomegaly present in both CP strains examined for this sign in young mice (Table 4) was not present in strains (N50, N56 and N145) which were producing experimental subclinical mastitis in sheep (18). Splenomegaly, like the other observed signs, seems to be useful in differentiating very virulent strains such as Staph. aureus from strains of reduced virulence such as C-NS (Table 4). All the C-NS tested had been isolated from cases of subclinical mastitis, but few of them showed signs of virulence in mice, and most were avirulent. One C-NS (N50) isolated from mild clinical ovine mastitis, a confirmed capsulated strain of C-NS (19), failed to cause marked pathological changes by the intraperitoneal route, but caused acute purulent mastitis in mice, as it had done in experimental sheep mastitis. Capsule is believed to be one of the virulence determinants (1) of staphylococci.

Death of mice inoculated with only sterile filtrates was thought the result of staphylococcal exotoxins. The most virulent CP strains had been isolated from gangrenous ovine mastitis. The strain causing only one death and five ill mice was negative for clumping factor (CF) and positive in the tube coagulase test after 24 hours of incubation. The one CP of which the filtrate did not affect the health of mice was a CF and tube coagulase negative strain at first isolation, becoming positive after subculturing. This strain had been identified as atypical Staph. aureus by the Central Public Health Laboratory of the United Kingdom. Failure of the filtrates from C-NS to cause any deaths is suggesting either complete absence of exotoxins or production of only relatively small amounts. Filtrates of some of the strains of C-NS tested here had been found to be non-toxic for ovine mammary neutrophils in in vitro studies (20) and their inability to produce exotoxins was verified here in vivo.

The local palpable reaction that occurred within the first 24 hours post-inoculation of mammary glands under the teat base and the large number of glands with histopathological signs of involution indicated that C-NS were non-invasive (10) causing only a localised reaction. This local reaction could result in teat sensitivity or formation of an abscess at the teat base obstructing the flow of milk both discouraging the young mice from suckling. Absence of suckling may have resulted in enlarged ‘white’ glands engorged in milk, seen here in 10 mice, and previously described as of ‘marble appearance’ (11) thought to be associated with invagination of the gland by C-NS. This engorgement with milk started the involution of the gland causing both clinical and histological changes.

Apart from the one capsulated Staph. warneri strain (N50) and one Staph. haemolyticus strain (N148), none of the strains of C-NS studied here produced the variety or severity of lesions in the mouse mammary gland described as possible by others (11). The amount of inoculum injected in previous reports (11) appears to be rather large for a local injection possibly contributing to mechanical inflammatory response which was absent here as result of considerably smaller inoculum.

Of the 45 strains of C-NS used in the present study 38 were the same for both adult and young mouse inoculations, but there is very little agreement between the two models with regard to strain and species virulence. Fourteen of these strains were related epidemiologically. They were isolated from consecutive samplings of the same sheep mammary gland, but varied in virulence between inoculation routes and also within the same route. The variation and pleiotropy observed in C-NS virulence is limiting the success of the mouse model. The process of screening large numbers of strains would become very laborious and expensive using the mouse model regardless of route.

However, differences between C-NS and CP strains in pathogenicity and invasiveness are an indication that C-NS could be good vehicles of Staph. aureus virulence determinants that could then be studied with any of the mouse models used in these experiments. Additionally, exoproteins seem to be important in the virulence of staphylococci and their absence in filtrates from C-NS could explain the low virulence of these organisms. Future work will need to utilise larger numbers of test animals and complicated statistical procedures for evaluating large quantities of dissimilar data. The mouse model is of limited value for studying the pathogenicity of strains of C-NS isolated from sheep milk and should not be seen as a convenient and affordable alternative to clinical experiments involving sheep.

Acknowledgments
Sincere thanks to Glaxo-Wellcome, Ltd. for the supply of mice and the preparation of pathology sections, and to Professor J. E. T. Jones for his encouragement and financial help.
References

World-wide Biomedical Science day

April 15th

World-wide Biomedical Science day is April 15th, not June as reported in Med Tec International No. 2 1998.

The theme for 1999 is Biomedical Science - the key to the control of Diabetes.
Minutes of the 54th Annual General Meeting Held at the Palmerston North Convention Centre, Palmerston North on Wednesday 2 September 1998

Chairman
The President (Ms S Gainsford) presided over the attendance of approximately 48 members.

Apologies
Motion:
Moved T Mace, seconded W Wilson
That the following apologies be accepted:
D Reilly
I Campbell
P Sarcich

Proxies
A list of 5 proxies were read by the Secretary.

Minutes
Motion:
Moved R Siebers, seconded T Mace
That the Minutes of the 53rd Annual General Meeting held on Wednesday 27th August 1997 be taken as read.

Motion:
Moved G Moore, seconded J Sheard
That the Minutes of the 53rd Annual General Meeting held on Wednesday 27th August 1997 be accepted as a true and correct record.

Business Arising
Membership
In 1997, 4th Year BMLS Students were offered free membership for 6 months and a number have elected to remain members.

Remits
Motion:
Moved T Rollinson, seconded T Mace
That Policy Decision Number 1 be reaffirmed.

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

Motion:
Moved T Rollinson, seconded R Siebers
That Policy Decision Number 2 be reaffirmed.

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

President’s Report
Motion:
Moved S Gainsford, seconded R Hewett
That the President’s Report be received.

Annual Report
Motion:
Moved T Mace, seconded G Moore
That the Annual Report be received.

Motion:
Moved W Wilson, seconded E Willis
That the Annual Report be adopted.

Financial Report
Moved T Rollinson, seconded A Paterson
That the Financial Report be received.

Dr McKenzie extended thanks to the NZIMLS for the donation to the PPTC which goes towards quality assurance for 22 hospitals in the Pacific and Asia.

Motion:
Moved T Rollinson, seconded C Campbell
That the Financial Report be accepted.

Election of Officers
The following members of Council were elected unopposed:

President S Gainsford
Vice President A Paterson
Secretary/Treasurer T Rollinson
Region 1 Representative S Benson
Region 2 Representative T Mace
Region 3 Representative C Kendrick
Region 4 Representative G Moore
Region 5 Representative L Milligan

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

Qualified Technical Assistant Awards
Clinical Biochemistry Kyle Jepsen, Medlab Hawkes Bay
Medical Cytology Christine Searle, Medlab Bay of Plenty
Haematology Leon Fruean, Diagnostic Laboratory
Histology Silas Harrison, Middlemore Hospital
Immunology Gordon Sutton, Medlab South
Microbiology Bharati Thaker, Diagnostic Laboratory
Transfusion Science Kylie Pearce, Medlab Southland

Specialist Certificate Awards
Medical Cytology Sharda Lallu, Wellington Hospital
Haematology Terrence Taylor, Dunedin Hospital
Transfusion Science Helen Muir, Dunedin Hospital

Journal Award
Best Review/Original Article Linda Pinder, Auckland Regional Blood Service
Honoraria

Motion:
Moved T Rollinson, seconded R Siebers
That no honoraria be paid.

Auditor

Motion:
Moved T Rollinson, seconded A Paterson
That Hillsen, Fagerlund and Keyse be appointed as the Institute’s auditors.

General Business

Annual Scientific Meeting

Questioned the outcome of the ASM survey. Council have decided to remain with having an ASM each year, but as from the year 2000, the ASM will be held over a weekend.

Noted that Council will be having more discussions with the SIG Convenors with regards to SIGs and Organising Committees working together on the ASM scientific programme.

Concern was expressed that the Biochemistry SIG is not running. Council have addressed this issue and have appointed a convenor and acknowledged others who are willing to have input into this SIG.

PRESIDENT’S REPORT ON THE NZIMLS 1997-1998

Last year I spoke of my concern at the financial situation of the NZIMLS. Happily this year we made a substantial surplus of $30,113. This is mainly due to a conference profit, increase in subscription, a smaller Journal deficit and the Council operating the Institute on a strict budget. The Journal remains under review.

The new Fellowship has been successfully introduced with 2 applications this year.

I have met with other health professional associations in Wellington to discuss items of mutual concern; the main issues have been regulation, the funding of clinical training and being represented on health committees.

The Council has made a number of submissions to Health Authorities on the funding of laboratories. Unfortunately as soon as a submission was made, the person or group it was made to seemed to change. The latest submission was to the Health Funding Authority asking that the NZIMLS be represented on the advisory group of the Primary Referral Laboratory Services and insisting that to be funded, laboratories be accredited according to National Quality Standards and Near Patient Testing be subject to the same standards.

A major goal was achieved. This was the issuing of a membership folio to all members of the Institute to keep their NZIMLS addresses, diary, rules etc in. New and updated information will be sent out regularly in the Journal to add to the folio. This could not have been achieved without sponsorship by Biolab Scientific. We are very grateful to John Knowles who agreed to the sponsoring of the packs, to Kirstie Baker who helped design them and to the rest of the Biolab Scientific team for this opportunity to improve services for our members.

A free 6 month introductory membership of the NZIMLS was made to 4th year BMLS graduates in an effort to encourage them to join their professional society and three have since joined.

The Council met with those scientific companies who are our major sponsors and surveyed members regarding the Annual Scientific Meeting and Special Interest Group meetings. We are now discussing changes to be made to these meetings.

The Special Interest Groups have again made major contributions to the continuing education of medical laboratory scientists, notably Histology, Microbiology and Transfusion Science who held seminars and Haematology who held a workshop and produced a document on ‘Standardised Reporting of Haematology Laboratory Results.’

I thank the Council for their support and for the time they have spent improving the NZIMLS. Thank you to Pip Sarcich who has not stood for re-election. Pip looked after Awards and brought fresh ideas on sponsorship and awards to the Council for consideration.

At the end of the last years AGM a member commented that they did not think the Institute should look inwards but use its money to advance the profession. At the time I still thought we had to be careful but I was mindful of his comment. Now we can move forward and I am sure that in the next year members will see changes that will have major benefits for the NZIMLS.

Shirley Gainsford
Examination Audits

This will be initiated in 1999 for NZIMLS examinations. One discipline per year will be audited for all levels of examination, ie QTA, Specialist, Fellowship.

The audit will consider the examination questions, candidate marks achieved and feedback to members following the examination.

The membership will be notified annually, via the Examination Liftout published in the Journal, of which discipline is to be audited in that year.

Fellowship

Two dissertations in Microbiology are currently being prepared.

**REMINDER**

Medical Laboratory Scientists who hold a Specialist Certificate are exempt from the Part 1 Examination of Fellowship for a limited time only. The final date for application under 3.12 of the Fellowship Regulations is March 31st, 2000.

Biochemistry SIG

Has a new Convenor - Trevor Walmsley, Canterbury Health Laboratories, Christchurch.

Nicky Thomas, Daphne Fairfoot and Tony Mace have expressed their interest in being involved.

Biochemists - support your SIG. Four is a small team to cover the country.

SIGs/Council

SIG Convenors are to be invited to meet with Council at their meeting, 23rd November in Wellington.

If you have items for the agenda of this meeting, please forward it to your SIG Convenor or to the NZIMLS Executive Office.

Membership Fees

Financially, the Institute continues to consolidate its financial position.

Special thanks must go to our Treasurer, Trevor Rollinson and our Executive Officer, Fran van Til for their careful monitoring and astute budgeting; to Council for reducing the number of meetings per year and also to the Wellington Conference (1997) for its profit contribution.

Council will continue to monitor closely the Institute’s financial position.

All members are encouraged to utilise the membership form in their membership folio and encourage non members to join their professional body and increase their personal opportunities for professional growth.

Health Funding Authority (HFA)

Submissions have been made to the HFA aiming to get representation of our profession onto the Laboratory Services Advisory Group along with Pathologists, Physicians, Managers and HFA representatives.

MLTB

Appointments to our Registration Board (MLTB) will be made soon. As is government policy, this must include lay people. If you have suggestions of informed, intelligent people who would be appropriate for ministerial (MOH) appointment to the MLTB, please contact the Executive Officer in the first instance.

Degree Courses

The NZIMLS and MLTB continue to monitor our professional courses at the University of Otago, Massey University and Auckland Institute of Technology.
Check out the Scientific topics:

- all labs have received at least two announcements to date
- all members received the latest brochure in their August Journal.

Updates available on the Internet
www.exevents.co.nz

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INSTITUTE BUSINESS

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Southern Community Laboratories, Dunedin

Council
Simon Benson, Chris Kendrick, Les Milligan
Tony Mace, Grant Moore

Executive Officer
Fran van Til
P.O. Box 3270, Christchurch
Phone/Fax (03) 313-4761.
E-Mail: exeevents@ihug.co.nz

Please address all correspondence to the Executive Officer, including Examination and Membership enquiries.

Editor
Rob Siebers
Dept. of Medicine, Wellington
School of Medicine, P.O. Box 7343
Wellington South.
E-Mail: rob@wnmeds.ac.nz

Membership Fees and Enquiries
Membership fees for the year beginning April 1, 1998 are:

For Fellows – $101.40 GST inclusive
For Members – $101.40 GST inclusive
For Associates – $48.10 GST inclusive
For Non-practising members – $42.20 GST inclusive

All membership fees, change of address or particulars, applications for membership or changes in status should be sent to the Executive Officer at the address given above.

Members wishing to receive their publications by airmail should contact the Editor to make the necessary arrangement.
New Products and Services

Fast and Accurate Evaluation of Enzyme-Linked Immunospot Assays
Carl Zeiss Vision announces the release of a new Software and Hardware System for automatic analysis of ELISPOT (Enzyme-Linked Immunospot) assays.

ELISPOT is a new and sensitive method of detecting and quantifying of individual T-lymphocytes which generate cytokine spots as a result of antigen contact.

The ELISPOT method can be used in all areas where ELISA measurements are performed, with ELISPOT offering a significant increase in sensitivity of up to 200 times.

The KS ELISPOT Software controls a motordrive stage, scans Microtiter plates at low and individual wells at high magnification, and performs autofocus and snapshots of cytokine spots for evaluation. The Software is designed to control a fully motorised Axioplan 2 Research Microscope for image acquisition and requires a PC with pen­­tium processor for fast analysis and evaluation.

For more information please contact:
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9-15 Davis Crescent
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Phone: (09) 520 5626
Fax: (09) 520 5619
email: info@zeiss.com.au
web: http://www.zeiss.de

ProSpecT Campylobacter Microplate Assay
The enteropathogenic bacterium Campylobacter jejuni is recognised as one of the major etiologic agents of acute diarrhea in humans. Infections cause diarrhea which may be watery and contain blood, usually occult, and faecal leucocytes. Other symptoms are fever, abdominal pain, nausea, headaches and muscle pain. The illness occurs 2-5 days after ingestion of contaminated food and can last 7-10 days.

Diagnosis of Campylobacteriosis infections presently rests upon isolation and cultivation of the organism in enrichment broth and selective media containing a variety of antibiotic supplements in a microaerophilic atmosphere of 5% oxygen and 10% carbon dioxide. Isolation can take from 2 days to a week. In addition Campylobacter frequently dies in transport and should ideally be cultured within 2 hours.

The new Alexon-Trend ProSpecT Campylobacter Microplate Assay is a solid phase immunoassay for the detection of Campylobacter Specific Antigen that detects pathogenic strains of Campylobacter in less than 2 hours with only minutes hands-on technologist time meaning same day or quicker diagnosis for the most common bacterial enteric pathogen. Viable bacteria are not required. The ProSpecT Campylobacter assay has a common format with other Alexon-Trend assays meaning several different assays eg. Giardia, Cryptosporidium, C.difficile, STEC and Campylobacter can be run at the same time, significantly reducing labour costs. Microwells are breakaway, making the kit suitable for smaller runs also, and reagents are ready to use.

Early detection means early treatment, quicker return to work and a better chance of tracking sources of infection.

For further information on the new ProSpecT Campylobacter Kit please contact Ngaio Diagnostics Limited at Fax 03 548 4727 or e-mail ngaio@xtra.co.nz.

ProSpecT Shiga Toxin E. coli (STEC) Microplate Assay
Shiga toxin-producing E. coli (STEC) strains have been recognised as important etiologic agents of diarrhea. They are also known to cause serious outbreaks and sporadic cases of life-threatening haemorrhagic colitis and haemolytic uremic syndrome (HUS).

E.coli 0157:H7 is the most frequently identified STEC serotype and can be isolated and identified by most clinical laboratories. However, at least 50 serotypes of E.coli, in addition to 0157:H7 have been associated with the production of cytotoxins and development of HUS and/or haemorrhagic colitis. If your laboratory is screening all bloody stools for E.coli 0157 you may be missing 45% of the Shiga toxin-producing E.coli infections that can cause life-threatening haemorrhagic colitis and haemolytic uremic syndrome (HUS).

ProSpecT Shiga Toxin E. coli (STEC) Microplate Assay is an immunoassay which allows direct detection of Stx1 and Stx2 toxins in stool specimens or in broth enriched cultures of the organism.

The assay has a common format with other ProSpecT enteric assays which means a streamlined procedure promoting workflow efficiency and increased productivity. Test results may be read visually or spectrophotometrically, negatives are clear and positives are distinct. There is no reagent preparation necessary, microwells are breakaway
for added flexibility and kits have a long shelf life. There is no cross-reactivity with Pseudomonas aeruginosa.

For further information please contact Ngaio Diagnostics Limited, P.O. Box 4015 Nelson South, e-mail ngaio@ixtra.co.nz

Market Announcement
Labsupply Pierce (NZ) Limited and Crown Scientific Pty Limited, Australia have come to a new and exciting agreement for Labsupply Pierce to market and distribute the product offering of Crown Scientific in the New Zealand market.

The agreement offers Labsupply Pierce with an additional range of new products which includes world-leading technology products from companies such as B. Braun Biotech International, environmental products from Casell and Palintest, vacuum technology from Plab, tissue culture plasticware from Iwaki, QVF industrial glassware, colour measurement products, solvent recovery / recycling products, plus a wide range of consumables and equipment products.

Labsupply Pierce effectively will take over the New Zealand operation of Crown Scientific Limited and they will now be able to offer the comprehensive Crown Scientific Catalogue which is available in hard copy, in full colour of over 900 pages, or in CD-ROM format.

For our valued customers in New Zealand, this innovative agreement means a valued addition of specialised products for their market, improved access to products to guarantee faster deliveries, a wider selection of consumables and general products and genuine savings by using the combined purchasing power of both organisations.

Mr Gareth Pryme, Manager of Crown Scientific NZ Limited, will transfer to Labsupply Pierce (NZ) Limited as of the effective of this new venture which is Monday, 31 August 1998.

For more information contact: Labsupply Pierce (NZ) Limited
127 Sunnybrae Road, Glenfield
Auckland, New Zealand
Telephone: 09 443-5867
Fax: 09 444-7314
E-mail: labsupply@labsupply.co.nz

New Zealand Internet Medical Software Launched
An innovative, New Zealand-developed medical software system, which will ultimately enable doctors to have access to patient laboratory results through the Internet, has been launched by Auckland’s Delphic Medical Systems. Delphic, which is already a major exporter of New Zealand medical software, anticipates considerable offshore interest in its new product.

Deliciously named ‘Eclair’ (Electronic Clinical Information Repository), the electronic suite of products for pathology laboratory and radiology reporting has been launched in two New Zealand public hospitals in Christchurch and Palmerston North. In both cases, the product is a first step towards a complete electronic environment where all patient information is held, entered, updated and ordered on computer.

Eclair will mean that lab and radiology results for patients are always available to the doctor needing them – through computers on wards, instead of through a cumbersome paper based system. Hospitals will see lab results the moment they are done, instead of hours or days after ordering.

“Duplication of testing in the hospital environment is reduced, because each doctor dealing with a patient knows what’s been ordered, and which conditions have been reported on,” says Delphic’s Business Development Manager Mark Cox.

In one case – Palmerston North Hospital – Eclair is implemented on an ‘Intranet’, a private, internally based information web, with access provided to key people within the hospital. Eclair on the Web as it is known, uses a network browser to provide easy access, and an easy to understand means of finding information for appropriate users. In Christchurch (Canterbury Health), Eclair has been placed on PCs in a Microsoft Windows based environment, using an internally based server to store data, and networked PCs to access it. It is known as Eclair on Windows.

But the concept behind Eclair means that it is capable of operating across the Internet. Many, geographically spread medical parties will potentially be able to access information on it through the Internet, using email and tight security protocols.

For GPs in the community, often waiting for patient information from the hospital, and for specialists, the Internet access will be a boon.

“In the future, community based doctors will be able to access laboratory and radiology testing information on their patients from the local hospital. Equally, the local hospital may be provided with historical testing records held by GPs” says Mr Cox. “This sort of flexibility is fantastic for doctors, who find it easy to use and time saving.”

A further benefit with Eclair is that multiple parties can interface with it to provide lab information, no matter how disparate their systems.

“A lab or specialist based outside the hospital can feed clinical testing information in, even from other parts of the country,” says Mr Cox. The ability to match different types of information and feed them into the one system is regarded as remarkable. To achieve it, Delphic used an American standard coding scheme for pathology and radiology test codes which cross references all test information.

Two software pioneers within Delphic Medical Systems’ Auckland based organisation – company director Roger Seaton and developer Dave Fallas – spent four years developing Eclair in New Zealand. Delphic has been responsible previously for the development of Palms Anatomical Pathology software, and other Delphic systems, which are now installed in over 50 sites around the world, including Canada, the United Kingdom, Hong Kong, Holland and Brunei.

Delphic and other hospital commentators regard Eclair as ahead of anything else in the world at the moment, and at a price lower than most other software serving the requirement to move clinical information into an electronic environment.

“Hospitals are needing to move all their records, information, diagnostic and treatment systems into an electronic environment. But this is enormously expensive,” says Mr Cox. “What we’ve done is take one aspect of their needs – pathology and radiology lab testing – and developed software which allows them to do this for a realistic, New Zealand price.”

Unlike many other medical software systems, Eclair comes with an in-built audit trail system as an extra level of security. That means, all users are tracked and traced, and the information they want noted for future review, as an extra check.

Eclair has also been piloted in Palmerston North and Christchurch hospitals so that high levels of security protocols are put in place, and special employment obligations are notified to potential users.

“Security is important. But it’s also important to note that this information is the same information that was provided with easy access on paper within the hospital or to other medical parties outside it. If anything, our system has tightened and focused use requirements,” says Mr Cox.

Delphic will begin marketing Eclair as the first New Zealand Internet based clinical information system, to hospitals around the world.
New Agency from Biolab

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ROUTINELY
PROCESSED
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- Provides sensitive and specific staining, superior to clone 1A6. The superior specificity of clone PgR 636 means that there is no cytoplasmic staining as with clone 1A6. Under optimal conditions, clone PgR 636 can be diluted up to 1:400 with LSAB2 and up to 1:1200 with LSAB+. This product is an excellent addition to an extensive line of breast marker antibodies.

DAKO Progesterone Receptor, PgR 636. Product code M3569.

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- Detects the Melan-A/MART-1 antigen in paraffin sections. Melan-A is a cytoplasmic protein and is expressed in the majority of human melanomas as well as melanocytes. Melan-A, clone A103, has been shown to be a more sensitive and more homogenous melanoma marker than HMB45.


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- Reacts with both normal and neoplastic hepatocytes. Highly specific for liver cells, detecting more than 85% of hepatocellular carcinomas. Gives a distinct, granular cytoplasmic staining of hepatocytes. It fails to react with a variety of other human malignancies excluding some gastrointestinal malignancies.

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For additional information call Med-Bio Enterprises Ltd. 0800 733 599.
Tenth Annual
NICE WEEKEND

A Transfusion Science educational opportunity organised by the TSSIG at Wairakei on 14-16 May 1999.

The NICE Weekend (National Immunohaematology Continuing Education) is an educational meeting for all people working in Immunohaematology and/or blood services. This year will be the 10th anniversary, so you can expect it to be a particularly NICE weekend and something special in the way of prizes. As usual it will be held at the Wairakei Resort Hotel.

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

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

• two nights (Friday 14 May and Saturday 15 May) accommodation on a share twin basis;
• continental breakfast, morning and afternoon teas, and lunches on Saturday and Sunday;
• dinner on Saturday night;
• transport costs will be your own responsibility.

Accommodation on other nights and other meals can usually be arranged directly with Wairakei Resort Hotel. This is also your own responsibility.

Please plan to arrive at the venue on Friday evening, as we have a full programme planned.

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

You will notice one new feature about the registration forms this year - FAXed confirmation of your registration. Because participant numbers are limited to the first FIFTY registrations we will fax your application form back to you on receipt, to let you know that your registration has been successful. If you don’t hear from us we have not heard from you.

If you have any questions contact Sheryl Khull, phone: 06 350 8013 or e-mail: sherylk@midcentral.co.nz

Surfing on Blood

With the increasing availability of the Internet in laboratories and homes, more and more information is becoming accessible to more and more people. Listed below are just a few of the web sites that may be of interest to anyone working in Transfusion Medicine or Blood Donor service. Many of these sites will contain links that you may follow to access more sites covering related topics. Happy surfing!!!

http://www.aabb.org/ - American Association of Blood Banks

http://www.infotrieve.com/freemeline/- Medline
http://www.americasblood.org/- America’s Blood Centres
http://www.pleasebewebblood.org/selfdoneyt.htm - American Red Cross site about autologous transfusions
http://www.blooddonor.org.uk/- national blood Transfusion Service (U.K.)
http://www.lisasem.co.uk/blood/bldfacts.html#From-Some-facts-about-blood-and-blood-donation
http://www.amazon.com - on-line bookstore which includes many books on transfusion medicine (best to wait until the exchange rate is better before shopping here though)
http://www.1iammed.com - DiaMed
http://www.bloodtransfusion.com/ - Questions and answers about blood transfusion
http://www.pall.com/ - PALL blood filtration page
http://www.iccbla.com/internationalsocietyofbloodtransfusionshort.htm - International Society of Blood Transfusion
http://www.healthworks.co.uk/hw/loss/scot.html - Scottish National Blood Transfusion Service
http://medicine.org.hk/hkabthtm - The Hong Kong Association Blood Transfusion and haematology
http://www.sanguin.com/ - The BTDS III Blood Transfusion Data System
http://www.redcross.org.hk/Blood/ - Hong Kong Blood Transfusion Service
http://www.medhelp.org/glossary/new/gls0740.htm - Blood Transfusion Reactions

Companies:
http://www.gammabio.com = Gamma Biologicals
http://www.pall.com/options/index.html - ‘Avoiding transfusion complications,’ a specific part of the Pall site.
http://www.morgamuf.com - Ortho Diagnostics (Johnson & Johnson) a comprehensive history of Rh HDN prophylaxis (obviously tied ultimately to the use of RhoGam!).

Journals useful for articles:
http://www.gmj.com - The ‘British Medical Journal.’

Transfusion Science Video Tapes

A series of video tapes is available for Transfusion Science revision. This series was collated in 1996 by the University of South Australia as part of a lecture programme, in collaboration with the Transfusion Science Special Interest Group of South Australia and CSL Biosciences. The 22 lectures were taped, two per video, and study questions were also provided.

http://www.redcross.org.hk/Blood/ - Hong Kong Blood Transfusion Service
These videos are available for a two week loan period from NZBS Southern Region
Box 4156
Christchurch
Attn: D Whitehead/R Hawes
The videos will be available at the 1999 NICE weekend.
For further information please contact Diane or Rosie

Lecture titles are as follows

Clinical Update 1996 - Transfusion
1 NATA, TGA, GMP, GLP and ISO9000
   What does it all mean?
   Dr Ross Savas (Red Cross BTS)
2 Current best practice in pretransfusion procedures
   Mr I Williams (IMVS)
3 RBC Antigens 1, Rh
   Mr D Roxby (FMC)
4 Transfusion in critical care
   Dr A Holt (FMC)
5 RBC antigens 2, K, Fy, Jk and other clinically significant antigens
   Mr D Ford (NBGRL)
6 Transfusion medicine in the 90s Beal (Red Cross BTS)
7 RBC antigens 3, ABO, Le, P
   Mr E Tocchetti (IMVS)
8 Triggers for transfusion
   Dr J Lloyd (IMVS)
9 Medico-legal and ethical aspects of transfusion practice
   Prof R Beal (Red Cross BTS)

Biochemistry

Special Interest Group

Convenor: Alison Buchanan
Clinical Biochemistry
Main Building
Auckland Hospital
Ph: (09) 307 4949
Ext: 7553
Fax: (09) 307 4939

Yes it is still alive!!

It is with great pleasure that the convener and committee members of the Biochemistry Special Interest Group introduce ourselves.
BSIG committee members.
Tony Mace Waikato Path Lab
Ph: (07) 834 118
Trevor Rollinson Southern Community Laboratory
Ph: (03) 477 6981
Rob Siebers Wellington School of Medicine
Ph: (04) 385 5999 Ext: 6838
Email: rob@wnmeds.ac.nz
Nicki Thomas Radiometer Pacific
Ph: (09) 573 1110
Email: nict@clear.net.nz

BSIG Annual Seminar
In the beginning of 1999, we would like to hold an annual Seminar. Ideally, we would like to get some feedback from you, so that we can organise a meeting that will;
- be informative, with material that you want to hear about,
- be in a venue, that the majority of you can get to, and
- be at a time that is convenient for you.
Do you prefer Rotorua, Taupo, or Nelson?
Are the school holidays easy or hard for you to arrange time away from the laboratory (and family)?
Perhaps you are presenting at another meeting between now and then, so will have something prepared already?!?
Get your thinking caps on and get back to any of the BSIG members with your feedback.
We look forward to hearing from you all.
Convener: Mr Trevor Walmsley
Canterbury Health Laboratories
PO Box 151
Christchurch
Ph: (03) 364 0300
Fax: (03) 364 0750
Email: trevorw@chhlth.govt.nz
**NICE WEEKEND**

14 - 16 May 1999

A Transfusion Science education opportunity organised by the TSSIG

Please register me for the 1999 NICE Weekend

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A brief abstract of your presentation must be forwarded by 17 April 1999.

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I enclose a cheque, made out to "NICE WEEKEND" for the amount of: $

Applications received after Friday 17 April 1999 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 Sheryl Khull, Transfusion Medicine, Palmerston North Hospital, Private Bag, Palmerston North, before 17 April 1999.

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

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HSIG Seminar – 1999
Introduction to Immunophenotyping and Molecular Haematology

Date: 19-20 February, 1999.
Venue: Dept of Molecular Medicine, School of Medicine, Auckland Medical School.
Duration: One and half days.
Participants: Numbers will be limited to 40 to allow for laboratory demonstrations.

The aim of the seminar is to introduce the topics to medical technologists from laboratories where these tests are not performed or those from larger laboratories who work in other areas of haematology. The programme will consist of a series of lectures from experts in the field and a visit to the Molecular Haematology and Transplantation Laboratory which is sited in the Auckland Medical School. Demonstrations of flow cytometry and some molecular techniques will be included. The seminar will also provide an introduction to workshops and scientific sessions at the 1999 South Pacific Congress.

Topics: Immunophenotyping
1. Techniques – Flow cytometry
   - Immunocytochemistry
   - Specimens (blood, bone marrow, tissues, FNA, fluids)
2. Current applications – Acute leukaemia
   - Chronic lymphoproliferative disorders

Topics: Molecular Haematology
1. Techniques – PCR
   - RT-PCR
   - Southern blotting
2. Current applications – Prenatal diagnosis and carrier studies (Haemophilia A and B, Thalassaemia)
   - Chromosome translocations
   - Haemochromatosis
   - Thrombotic disorders (Factor V Leiden and Prothrombin variant)
3. Recent developments / new techniques and applications

Convenors: Jan Nelson and Neil Van de Water, Molecular Haematology and Transplantation Laboratory, Auckland Healthcare and Dept of Molecular Medicine, Auckland School of Medicine.
Registration: Registration forms will be available in December from the convenors.

For Further Information Contact:
Jan Nelson: Tel 09-3737-599 ext 6381 Email: j.nelson@auckland.ac.nz
Dr Neil Van de Water: Tel 09-3737-599 ext 6329
Email: n.vandewater@auckland.ac.nz

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Biochemistry in Paradise
By Elizabeth Stephenson

I was privileged to spend 1997 as a volunteer at Vila Central Hospital (VCH) in Vanuatu, while my husband was employed as a volunteer with the United Nations Development Programme on a project in the country. Vanuatu is a developing country consisting of over 80 islands situated in the south Pacific. The Laboratory participates in the Pacific wide, WHO Collaborated Pacific Paramedical Training (PPTC) Centre's Quality Control Programme. These are run by WHO Collaborated Pacific Paramedical Training (PPTC) Centre's Quality Control Programme co-ordinated from Wellington, New Zealand. I had contacted Mike Lynch, Co-ordinator PPTC And Clare Murphy, Clinical Chemistry Tutor prior to leaving NZ, about opportunities to use my Laboratory skills while in Vanuatu. (I was on leave without pay for one year from the Biochemistry Unit, Canterbury Health Laboratories, Christchurch Hospital, where I had worked since 1985.) With a letter of introduction from Clare, I approached Helen Wamle, acting Principal Laboratory Officer, Vila Central Hospital Laboratory and was offered a part-time position (unpaid) for 4 hours per week. A job description was prepared outlining my position as advisor and trainer in quality control systems in the Biochemistry Laboratory. I was to help the 2 Biochemistry staff: Esau Kalfabun, Head Technologist Biochemistry and Raymond Seulie, Technologist. I was to report to Helen Wamle and VSO (British Volunteer Services Overseas) to assist with the development of the Biochemistry Laboratory, which was the second largest centre on another island.

VCH evolved from the combination of the Old French and British Hospitals (Vanuatu, prior to independence, then called the New Hebrides, was a condominium run dually by France and Britain). The senior Lab staff originated from the French Hospital, which was trained by French Nuns. French is still the preferred language amongst these staff, but they also speak their own island group language, Bislama, the pidgin English that unites all the island group languages, as well as English! The junior staff have been trained at Fiji School Of Medicine's, Medical Laboratory Technologist school are more confident in English. It certainly made for an interesting first few days with communication between and 9 pathology staff, occurring in all of the languages!

Upon starting in February, the biochemistry analysis was carried out using manual Immunoex and Sigma Test kits. After a thorough evaluation carried out by the Laboratory Advisor, 4 Vitros DT60 analysers were purchased with funds from AusAid (Australian Aid). These analysers were placed in 4 of the 5 hospital laboratories spread throughout the country. Installation was carried out along with in-country training in the analysers use and maintenance. Quality control charts (Levey-Jennings type) were supplied with the analysers and I then began training in their use and interpretation (at VCH only). Other projects I embarked upon were to advise and train in procedures for assessment of the micropipettes accuracy and precision. Also to institute the daily recordings of refrigerator and waterbath temperatures. I also helped with problem trouble-shooting in biochemistry.

As the year progressed the improved accuracy, precision and the test result turnaround time achieved with the new analysers resulted in an increased workload of nearly 300%. This as well as the improved accuracy and precision evidenced by the PPTC external quality control program results, produced a marked increase in staff morale and confidence. As time went by, staff began to reveal their thoughts and feelings on issues concerning the laboratory. One recurring problem mentioned was the generous but ill-conceived donations of laboratory equipment from overseas donors which are selected without consultation with laboratory staff or the Lab advisors (e.g. PPTC) and without consideration of the actual needs and ongoing operating costs. This caused much frustration with staff, as when the equipment failed it was costly to repair or replace parts. This equipment then sat, unused, cluttering up the benches. Staff were understandably reluctant to discuss these issues with the donor agencies or organisations for fear of 'biting the hand that feeds them.' As, every now and again they do receive equipment that is appropriate and has reasonable ongoing costs.

I found working in this Laboratory to be challenging in many dimensions, but as is often the case, intensely rewarding and fulfilling. I found it important to allocate a considerable amount of time to just observe the staff and their work environment and ask questions in a tactful and sensitive manner. Then to listen without judgement and only then begin to think how I could help in the most appropriate way for the situation. Only then could I begin to advise and train, all the while keeping it in mind that I could only advise, as the laboratory decisions rested with the laboratory staff. My initial sessions were a confusing and awkward time for me with conflicting impressions as to whether I was welcome. I later realised that I had made incorrect assumptions and following a meeting to discuss issues. I found that the hours I had chosen to work were unsuitable for the work routine. Working with cultures where English is the second, third or even in some cases fourth language can cause problems of understanding. Sometimes it felt like staff were pretending to comprehend to avoid embarrassment. This could be avoided with repetition of the original sentence in various ways and sensitive questions to check if I had been clearly understood.

My most rewarding experience was to observe results from training, when one of the Technologists, without assistance, successfully diagnosed and corrected a laboratory problem using deteriorating QC chart as the sole indication of a problem. This Technologist then of his own volition suggested this case for presentation of the importance of QC charts at an upcoming DT60 User Group Meeting for other laboratory workers throughout the country.

Contribution to Developing Countries
There are many other ways to contribute to developing nations if you are unable to volunteer. Supply of unused or excess posters, diagrams, textbooks (even if they are old editions), tourniquets; diamond markers, micropipettes, and glassware, (especially volumetric). Contact PPTC before anything gets thrown out!

Acknowledgments
I am very grateful for the opportunity given by Helen Wamle and the staff at Vila Central Laboratory. I hope that my contribution will continue to be useful. What I have learnt personally from this experience has been an unexpected bonus. I would also like to thank the Mr. Johnson Waabaat Director of Health, Vanuatu, for permission to publish this article.


**Pacific Profile**

**Name:** Helen Wamle Taleo

**Present position:** Acting Principal Laboratory Officer

- Vila Central Hospital Laboratory
- Port Vila
- Vanuatu

**Training and Qualifications:**

- 1970-71 Locally trained at the French Hospital Laboratory Port Vila.
- 1971-74 Technician at the French Hospital, Santo, Vanuatu
- 1974-80 Technician at Georges Pompidou Hospital, Port Vila.
- 1980-94 Senior technician, haematology, Central Hospital Port Vila.
- 1995-present Acting Principal Laboratory Officer.
  - Attended many national and international courses and workshops.

**Main interests in laboratory work:**

- Haematology,
- Blood bank, and
- Microbiology

**Highlight of my career:** Being a simple locally trained technician and reaching the top laboratory position in the country.

**What are the main issues facing laboratory work in Vanuatu?**

The need for a pathologist based at the Central Hospital.

- Stretching finances to cover the costs of a considerable increase in the number of laboratory tests performed.
- Obtaining local expertise to perform instrument maintenance.
- Establishing a cytology service.
- Implementing a laboratory staff structure to take the laboratory services into the new millennium.

---

**The Roomful of Straw**

Readers may recall the Grimm Brothers' Fairy Tale, Rumplestiltskin.

In this, and similar tales, an individual is imprisoned by an unreasonable persecutor who will release her if she can meet an impossible request.

- Each evening of her imprisonment, she must spin a roomful of straw into gold. Each morning the prisoner has completed the task.
- The roomful of straw assumes larger dimensions for each successive evening.
- Release can only be granted if the prisoner guesses correctly the name of her persecutor, (in this case, "Rumplestiltskin").

In the real world, I have observed the descent of this curse onto the unsuspecting laboratory scientist managing a Unit within a larger Laboratory (often, but not always, a Public Sector entity).

For the purposes of demonstration of this phenomenon in action, imagine a hypothetical "Business Unit" within a larger Laboratory business.

The Unit usually returns a significant profit for the Laboratory.

**Room 1**

Work volume is constant, price for the test is constant, staff is sufficient for the output of the Unit, overheads are covered, and a modest profit is made.

**Room 2**

Work volume increases, usually as a result of external marketing efforts, but also supported by the staff within the unit.

- Staff levels remain constant; but stress levels increase.
- Overheads increase. Price for the test remains constant. The Unit, despite increased workload, now runs at a loss.

**Room 3**

Overheads increase significantly, but the mechanism for allocation of overheads becomes obscure. The Unit may well be supporting a number of poorly performing sections elsewhere within the Organisation, but the Unit Head has no way of confirming this suspicion.

- Staff levels will be the same as Room 1, but stress levels will have increased arithmetically as staff realise that despite extra effort and input, the Unit is running at an even greater loss.
- At this point, the price for the test may increase, which will release some budgetary pressure; this respite will be offset by an increase in workload as marketing of the test increases.

Within the Unit, deteriorating morale and insufficient equipment will result in increased turnaround time and apathy towards clients.

**Room 4**

Test volumes continue to increase. Hopelessness permeates the Unit as it becomes obvious to staff that although revenue has increased substantially since Room 1, conditions within the Unit have deteriorated significantly.

- Staff begin to transfer to other positions within the Laboratory (if this avenue of escape remains open), or seek jobs with other similar Organisations.

**Room 5**

Loss of expertise results in deterioration of service. The customers may note that prices have increased, while quality has declined.

- Test volumes drop, the Unit is no longer seen as profitable, and becomes vulnerable to other providers.
- The skilled staff, responsible for the success of the Unit, have gone elsewhere.

Despair sets in.

I call this phenomenon "The Roomful of Straw".

The moral, both in the Fairy Tale, and also in the Real World, is that "straw is straw; but skills are Gold."

Informal discussions with other laboratory scientists, working in both medical and non-medical fields, have confirmed the phenomenon exists.

I invite comment from other Medical Scientists throughout the country to further confirm or challenge the validity of this previously unreported pattern of organisational behaviour.

John Aitken

The Princess Margaret Hospital

Christchurch
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Technology and Diagnostics
Ian Wright
European Marketing Director of Plasma Proteins
Dade Behring Diagnostics

Diagnostic Laboratories worldwide find themselves at a time of great change. The pace of change differs geographically and is under different but constant pressure. Technology plays a part in that change. Consolidation in the Laboratory is one way that we can alleviate these pressures. Consolidation is not the only answer. No one is yet advocating that science stands still and if a clinical and or cost benefit for a new test can be demonstrated, then it will be taken up by the clinical community. Sometimes tests need a more specialised detection method either because they are new or a special measuring technique e.g. nephelometry is required to get the full clinical utility from a test.

The modern diagnostic Laboratory needs to be efficient but, as far as it is possible, not compromising in terms of the quality of its output or its ability to shape medical practice through new and existing diagnostic tools.

Agricultural and Biomedical Applications from Recent Advances in Animal Cloning Technology
Dr David Wells (and HR Tervit)
Research Scientist
Reproductive Technologies Group
AgResearch
Ruakura Research Centre

Many exciting new opportunities for research, agriculture and biomedicine arise from recent advances in nuclear transfer technology. These advances have now enabled the production of cloned sheep and cattle from cultured somatic cells that have been obtained from either embryos, foetuses or, most notably, from adult animals. It is now possible to take differentiated cells from an animal and reprogramme them such that the normal sequence of gene expression commences once again from early embryo stages, to ultimately produce a genetically identical individual. The nuclear transfer process involves the removal of metaphase II chromosomes from an unfertilised egg and then the electro-fusion of a cultured cell to the egg cytoplasm. The cultured cell provides the donor nucleus containing the new genetic information. With access to large numbers of cells from cultured cell lines, this nuclear transfer process can be repeated many times. Following artificial activation of reconstructed one-cell embryos, approximately 30% develop to the blastocyst stage (around 130 cells) after seven days of in vitro culture. Following transfer to the reproductive tract of surrogate females, 10% of the cloned embryos may develop into offspring. Overall, these efficiencies are currently one-third those achieved following standard in vitro fertilisation in the bovine. It is expected that the present techniques will be improved upon, particularly as knowledge of nuclear reprogramming increases, and extended to other species.

In agriculture, cloning enables the rapid dissemination of superior animals from breeding flocks and herds to commercial farmers. This could be achieved through the controlled release of selected lines of elite cloned animals or alternatively, in the case of the sheep and beef industries, the cloning of elite ram or bull teams to use for natural mating could possibly replace artificial insemination. The use of cloning should be minimised in nucleus breeding populations, so as not to overly reduce the genetic variability necessary for continued selection of superior animals. Cloning could yield economic volumes of new agricultural products to rapidly meet changing market requirements and also generate more consistent quality products desired by consumers. Large sets of genetically identical farm animals will be used in research to reduce the genetic variability in experimental designs. The most immediate application of this technology, however, will be in conjunction with gene targeting. Very precise genetic modifications will be introduced to cultured cells growing in the laboratory and then nuclear transfer will be used to generate transgenic farm animals. Particular genes will be either introduced, subtly modified or even removed to result in a wide range of desired outcomes, which include: improved livestock production characteristics, conferred pest and disease resistance in livestock; production of pharmaceutical proteins in the milk of farm animals to treat specific human diseases; replacement of bovine milk genes with human equivalents making milk more nutritious for pre-term human infant formula; production of pigs whose tissues and organs are immunologically compatible with human patients preventing hyperacute rejection following xenotransplantation; and the production of animal models for specific human diseases to test new therapies. In addition, autologous cell based therapy (in some situations also linked with gene therapy) will benefit from the eventual understanding of the biology associated with the nuclear reprogramming of differentiated cells. Cells taken from the human patient will be firstly de-differentiated in vitro and then used to subsequently generate the specific cell types required to repair damaged or diseased tissue. In this way, the patient may generate their own therapeutic tissue for transplantation and thus, avoid the complications of immune rejection. In summary, 'Dolly' has opened many new possibilities that will ultimately benefit society in the future.

Genetic Testing for Stomach Cancer Susceptibility
Dr Parry Guiford
Research Fellow
Cancer Genetics Laboratory
Biochemistry Department
University of Otago

The identification of genes predisposing to familial cancer is essential not only for the clinical management of affected families but also for the description of the genetic events of tumorigenesis. Despite a declining incidence, gastric cancer remains a major cause of cancer death worldwide, and about 10% of cases show familial clustering. The relative contributions of inherited susceptibility and environmental effects to familial gastric cancer are poorly understood because little is known of the genetic events that predispose to gastric cancer.

We have recently identified mutations in the gene for the cell to cell adhesion protein E-cadherin in three Maori families. These mutations are responsible for an inherited susceptibility to the histologically diffuse form of gastric cancer. We have also identified E-cadherin mutations in two North American Caucasian families affected by the same form of cancer.
Knowledge of the E-cadherin mutation, which co-segregates with the disease in gastric cancer families, enables the identification of individuals who have an extreme lifetime risk (70%) of developing cancer. These people can be put on clinical surveillance programmes to detect developing gastric adenocarcinomas before they have invaded beyond the gastric mucosa. We have begun screening New Zealand gastric cancer families with known E-cadherin mutations. The presence or absence of the mutation is detected by either direct DNA sequencing of PCR products or allele-specific oligonucleotide hybridisation. Clinical and psychological issues associated with this testing will be discussed.


'Cancer Genetics Laboratory, Biochemistry Department, University of Otago, PO. Box 56, Dunedin.

'Mini Hauora Health Clinic, Mt Maunganui.

Mad Cows and Englishmen – Are There Lessons For New Zealand?
Professor Colin Wilkes
Institute of Veterinary Animal & Biomedical Science
Massey University

The first report of a spongiform encephalopathy resembling scrapie in a cow in England started a resurgence of interest internationally in the transmissible spongiform encephalopathies, which followed closely the emerging BSE epidemic in the United Kingdom. While the initial diagnosis of BSE relied on veterinary clinicians and pathologists, epidemiologists played a significant early role in elucidating the mode of transmission and predicting the likely future course of the epidemic given the application of recommended control measures. Some would still dispute that the prion hypothesis is an adequate explanation for the aetiology of these diseases but the work of molecular biologists has greatly expanded our understanding. From the outset there were concerns about the potential for BSE to spread through the food chain into the human population and current evidence indicates that this has occurred although, as yet, only a very small number of cases have been identified.

The epidemic has had broad ramifications including concerns about the safety of food and biomedical products; international movement of animals and animal products including embryos and semen; and the challenge to countries to devise convincing surveillance and testing protocols to demonstrate freedom from BSE and related diseases. There have also been important lessons about the ways in which the public and news media deal with emerging diseases. This is particularly apparent as decisions are made and ‘assurances’ given in the absence of complete information. The BSE epidemic is not the only emerging disease to have caused concern in the last decade nor is it unlikely that further ‘new’ diseases will emerge in the future. Perhaps the most important lessons are those relating to public education about the ways in which scientific investigations are conducted, the need to change opinions as new information is acquired and the acceptance that we do not live in a risk free world.

Amylin, Amyloid & Diabetes Mellitus
Garth Cooper
Professor in Biochemistry & Clinical Chemistry
University of Auckland

It is now more than ten years since pancreatic β-cells were found to secrete amylin, a peptide hormone originally discovered in islet amyloid deposits from patients with non-insulin-dependent diabetes mellitus (NIDDM). Increasing evidence has implicated amylin in disease mechanisms of both NIDDM and insulin-dependent diabetes mellitus (IDDM). This paper will critically review the evidence implicating amylin in the causation of NIDDM. In particular it will be shown how amylin forms islet amyloid and that fibrillar amylin damages islet β-cells. These lines of evidence implicate amylin as a central player in the β-cell failure of NIDDM.

Amylin is lacking in IDDM, and this deficiency has now been shown to influence the response of such patients to insulin therapy. Amylin co-replacement was predicted to improve blood glucose control without increasing the rate of hypoglycaemia, and thereby to decrease the rate of long term diabetic complications. Large scale clinical trials with pramlintide, a pharmaceutically stable analogue of human amylin, show that these hopes are borne out. Amylin therapy is likely to affect a major improvement in glycaemic control and complication rates in both IDDM and late-stage NIDDM.

Probe Diagnostics
Dr Sharon Muldoon
Manager of Probe Scientific Affairs
Abbott Diagnostics Division
Abbott Laboratories
Chicago

The commercialisation of nucleic acid amplification assays for several infectious disease pathogens has signaled the successful transition from a research technology to a diagnostic tool for the clinical laboratory. Their availability has enabled the clinical community to go beyond better detection and radically change the management of several of our current most serious infections. In the example of Chlamydia trachomatis diagnosis, amplified probe testing has not only improved the detection of infected patients, it has raised the possibility of significantly lowering the prevalence of infection by screening large segments of the population. The performance and unique features of nucleic acid technology (NAT) which have enabled this re-statement of Chlamydia management will be discussed. Similarly, in the management of HIV infection, quantitative viral load assays have been developed using RT-PCR, which are an integral part of the powerful new highly active anti-retroviral therapy regimen. For the future, developments are currently under way to provide probe technologies with improvements in automation throughput and ease of use. Finally, with the potential for pharmacogenomics testing, laboratory testing and the practice of medicine will become even more closely linked.

Disease Management and Diagnostics
Ian Wright
European Marketing Director of Plasma Proteins Behring Diagnostics

Changes and advances in vitro diagnostics come about in two main ways: either through the development of a complete new parameter which changes clinical practice, or a well established test finds a new value through changes in clinical practice or demonstration of a cost benefit. Using recent examples in the areas of cardiology, transplantation, nutrition and renal disease it is hoped to demonstrate some new areas of interest in medical diagnostics.

Automating PCR for Molecular Diagnostics
John Mackay
Roche Boehringer Mannheim

Since the first publication describing the Polymerase Chain Reaction (PCR) in 1985, the increase in the number of reports and applications has behaved much like the reaction itself: exponentially! Whilst
DNA/RNA amplification strategies such as PCR have shown the greatest sensitivity and specificity for many clinical applications, it is only recently that these methods have been employed by laboratories with those other than trained molecular biologists. Much of this has been due to unknown handling procedures, no standard methods available, fear of contaminating assays and general lack of training available. Here we will describe the developments of the AMPLIFIRE™ methodology for clinical diagnosis by PCR, as well as the new introduction to New Zealand: the COBAS AMPLICOR™ for automated amplification and detection on the one instrument. Future work (AMPLIPREP™, TaqMan®) will also be discussed.

The Thrombolytic Assessment System (TAS™):
A Laboratory's Tool for Rapid Interventional Testing
Julie Leumas
Cardiovascular Diagnostics, Inc.
Raleigh, NC
USA

Advances in diagnostic and interventional procedures and the development of new therapeutic agents have prompted innovative approaches to laboratory medicine and the services the laboratory offers to its customers. Patient-focused strategies have concentrated on reducing hospital stays and managing treatment on an outpatient basis. This evolution in healthcare challenges traditional laboratory medicine as the demand for decreased turnaround in test results increases. Conventional laboratory systems are cost-effective and efficient methods, which satisfactorily process most laboratory tests. In cases where rapid test results are necessary for immediate intervention, conventional laboratory methods, which rely on sample transport and processing, often are inadequate. One alternative to conventional laboratory systems is the point of care (POC) system which is designed to provide rapid test results at or near the patient's bedside. POC systems, in general, provide rapid information, are convenient to use in multiple healthcare environments, and may be the only viable solution for some patient monitoring.

The Thrombolytic Assessment System (TAS™) is a POC method for monitoring multiple coagulation parameters including the PT, APTT, and HMT™ (Heparin Management Test) as well as clot lysis associated with thrombolytic therapy. The system uses a novel technology based on paramagnetic iron oxide particles (PIOP) which move under the influence of an oscillating magnetic field. The PIOP are combined with dry reagents within a disposable test chamber mounted on a card. The card has a magnetic strip, which contains lot specific information used to initialise the analyser. An imprecisely measured whole blood or citrated whole blood sample is added to a sample well on the card, which triggers the test to begin. The analyser notifies the operator when the test is complete, displays the test result, and stores the result with lot specific information and sample identification. The software can be configured to require operator identification and quality control time and range lockout. All results can be downloaded to a printer or computer for further data management.

The TAS is considered an extension of the laboratory. It combines many of the software features commonly found on conventional systems with the convenience of POC systems. The TAS has been used in multiple hospital settings including warfarin clinics, cardiac catheterization, the OR and in the more traditional laboratory setting where test volume or personnel cannot support a larger analyser. It is particularly useful for monitoring anticoagulation therapy, and may provide a more accurate assessment of a patient's hemostatic potential when sample integrity is compromised as the result of delayed transport and processing.

Point of care systems contribute to current strategies and trends in healthcare. They are a useful tool for providing rapid interventional test results when conventional laboratory methods cannot address testing requirements. Increasingly, POC will become a more significant part of laboratory services and laboratory professionals should investigate those systems that provide quality results and effective data management. The TAS is one POC system, which is designed to meet the demand for rapid test results and maintain quality standards the laboratory expects.

An Impossible Case of Immune Thrombocytopenia Purpura
Dr Elayne Knottnebelt
Consultant Haematologist
MidCentral Health/Medlab Central

Immune Thrombocytopenia (ITP) is the most likely diagnosis in a patient with isolated thrombocytopenia. In the majority of cases the outlook is good, particularly in children who tend to recover spontaneously. In adults ITP may be more difficult to control, many requiring splenectomy. Response to splenectomy is usually excellent.

A case of a 42 year old man who presented seven years ago with ITP associated with red cell aplasia and a positive direct antiglobulin test is used to illustrate the extreme difficulty in treating refractory ITP and to demonstrate the wide variety of agents used.

Prothrombin 20210 Variant in New Zealanders with Thromboses
Catherine (Kate) Marson
Haematology
Medlab Central Ltd
Palmerston North

Familial Thrombophilia is a complex genetic disorder. Some of the genetic risk factors for venous thrombosis include deficiencies of Protein C, Protein S, and Antithrombin III, and Activated Protein C Resistance caused by the Factor V Leiden mutation. Individuals with more than one genetic defect are at higher risk of a venous thrombotic episode.

In 1996, a genetic variation was reported in the 3rd untranslated region of the Prothrombin (Factor II) gene, on chromosome 2. The presence of the G20210A variant has been reported to increase the risk for venous thrombosis by 2.8 (nearly 3-fold). The variant has been detected by PCR. A recent American study has also pinpointed the G20210A variant as a risk factor for Myocardial Infarction in young women, with the risk increased 4-fold. (Heterozygous Factor V Leiden increases the risk of MI by 2.5).

In the Hawke's Bay, a family has recently been identified as being affected by the Prothrombin G20210A variant. Three female members of the family have been tested by PCR and found to be heterozygous for the gene variant. Two of the women presented with symptoms, family studies were initiated after the second patient was identified. The third woman remains asymptomatic. All other thrombophilia screening tests were normal. Factor V Leiden was performed which was also normal.

There is no doubt that the Prothrombin G20210A variant is present in the New Zealand population. At what level it is present is not yet known. Increased ability to test for the abnormality may help to solve some previously unexplained thrombotic episodes around the country.

Evaluation of the Thrombolytic Assessment System (TAS™)
APTT Using Citrated Whole Blood
Mandy Wood (and Dr George T C Chan)
Department of Haematology
Greenlane (& National Women's) Hospital, Auckland

The TAS is a lightweight, portable blood coagulation analyser. The dis-
The TAS test cards contain dry reagent plus paramagnetic iron oxide particles. The paramagnetic iron oxide particles move in response to an oscillating magnetic field. The sample is drawn to the reactive chamber by capillary action. The sample dissolves the reagents and the paramagnetic iron oxide particles begin to oscillate. The movement is monitored optically by the analyser. Fibrin polymerisation causes significant restriction of movement of the paramagnetic iron oxide particles. This change in movement is detected as the end point of the test. The APTT test cards contain phospholipid derived from a chloroform extract of rabbit brain, calcium chloride and a particulate activator aluminium magnesium silicate. The characteristics of the TAS-APTT were compared to the APTT determined by the Behring Coagulation Timer (BCT) using Automated Organon Teknika APTT reagent.

**The Evaluation included:**

1. A normal range study. 20 samples with APTT values within the normal range were tested by both methodologies.
2. A patient sample study. 50 samples were tested by both methodologies. All 50 samples tested were obtained from patients receiving unfractionated heparin.
3. A precision study. One sample tested 20 times on the TAS.
4. An APTT heparin sensitivity study. Pooled normal samples were spiked with varying concentrations of unfractionated heparin and tested by both methodologies.

**The findings:**

1. The normal range study correlated well with the laboratory method. Laboratory normal range 25-37 seconds; TAS normal range 25-36 seconds.
2. The patient sample study showed that the TAS APTT results from patients on heparin were makedly shorter than the APTT results obtained by the laboratory. The TAS APTT did not appear to be as sensitive to heparin as the laboratory APTT. There was also an overlap of TAS-APTT values (from heparinised patients) with the normal range.
3. The precision study showed good precision. CV = 5.0%
4. The APTT heparin sensitivity study. The TAS APTT range at heparin concentrations of 0.2 - 0.4 U/ml was 55 - 80 seconds. Which is similar to the therapeutic range of 60-100 seconds currently in use at GLNWH.

**Conclusion**

Although the in-vitro heparin sensitivity curve for TAS-APTT determined on heparin-spiked specimens showed a linear relationship between the heparin concentration and the APTT. When patient specimens were used this linear relationship disappeared. The TAS-APTT is less sensitive to in-vivo heparin with overlap between patient results and the normal range. This together with the requirement of the system for citrated whole blood made the instrument unsuitable for routine use by ward staff.

**Evaluation of Specimen Handling Protocol for Neutrophil Alkaline Phosphatase Analysis**

Greg Pinnell
Haematology Department
Diagnostic Laboratory Auckland

**Introduction**

Neutrophil alkaline phosphatase is an enzyme, which is involved in the breakdown of foreign materials and organisms within the body. The level of neutrophil alkaline phosphatase is consistently altered in a number of disease states and is therefore a useful diagnostic tool. Problems may unfortunately arise with the accuracy of the result when the assay is to be performed outside the collection area. This could be due to inappropriate and subsequent loss of enzymatic activity over time.

**Methods**

In order to investigate this problem several different protocols for specimen handling before analysis were evaluated. These included variations in fixation of the blood as well as different storage and transportation temperatures.

There were three different specimen types evaluated: fixed blood smears, unfixed blood smears, and whole-heparinized blood. The temperatures investigated were: -20°C, 4°C, and room temperature. Prior to analysis the specimen moved through three temperature stages before analysis; storage prior to transportation, transportation, and storage after transportation. Temperature combinations were evaluated in regard to these three stages.

**Results**

From the analyses of variance performed on the data it was apparent that whilst the type of specimen used had a significant impact on the results, the storage and transportation temperatures had no significance.

**Discussion**

The method that was chosen, taking into consideration simplicity for the laboratory staff, was to make and fix the blood smears at the time of collection. The smears were then stored and transported at room temperature.

**Acute Promyelocytic Leukaemia: A Case Study**

Dr Bart Baker
Consultant Haematologist
MidCentral Health

A 22 year old student presented to her General Practitioner on 11 July 1997 with a two week history of bruising and menorrhagia. Her blood count revealed a haemoglobin of 128 g/L, white count 3.9 x 10^9/L, neutrophils 2.68 x 10^9/L and platelets 23 x 10^9/L. Coagulation profile revealed an INR of 1.3, APTT 31.6 seconds (normal 25-40) and a Fibrinogen of 0.35 g/L (normal 1.4-4.5).

By 14 July 1997 her platelet count had dropped to 14 x 10^9/L and she had a number of abnormal promyelocytes in the peripheral blood. Her Fibrinogen remained markedly reduced and her D-Dimer assay became positive.

A Bone Marrow Aspirate confirmed a diagnosis of Acute Promyelocytic Leukaemia and she was commenced on a combination of All Trans Retinoic Acid (ATRA) and intensive chemotherapy. She achieved remission following one cycle of chemotherapy and ATRA and received several cycles of consolidation chemotherapy according to the MRC AML-1 2 Protocol.

The biology and therapy of Acute Promyelocytic Leukaemia will be discussed with reference to this case.

**Molecular Mechanisms of Cancer Chemotherapy Resistance**

Dr Richard Isaacs
Consultant Medical Oncologist
MidCentral Health

A number of mechanisms define tumour sensitivity and resistance to cancer chemotherapy. We have been studying topoisomerase II, a critical nuclear enzyme that acts to maintain optimal DNA topology, enabling efficient nuclear function, particularly during mitosis. This enzyme is also now recognised as the critical molecular target for a number of commonly used anti-cancer drugs, with drug sensitivity often proportional to the degree of enzyme expression.

We have previously identified a region of the topoisomerase gene promoter that is critical in regulating topoisomerase II gene expression in different growth states. Our present work is to identify
whether there are mutations within this region, or elsewhere in the
gene, in breast and ovarian tumours, which may modify expres-
sion and determine drug sensitivity.

Richard J Isaac, Carina a Miles, Robyn Marston, Helen A
Turley, Kathryn M Stowell, Adrian L Harris

Estimation of Busulphan Levels in Bone Marrow
Transplantation
Grant Moore
Toxicology Section
Canterbury Health Laboratories

Introduction
A novel method is presented for monitoring the chemotherapy agent,
busulphan, which is used as a conditioning agent in bone marrow
transplantation. Concentrations determined from the loading dose are
plotted against time and the area under the curve is
used as the basis of therapeutic drug monitoring.

Methods
Patients are dosed with busulphan for conditioning prior to bone mar-
row transplantation at a rate of 1 mg/kg. Plasma samples are drawn at
30, 60, 90 and 360 minutes following the first dose of busulphan.
Samples are separated and, if not analysed immediately, the plasma is
stored at -20°C. Busulphan is derivatised by the addition of potassium
iodide and heating at 70°C for 40 minutes. After extraction into an
organic solvent the busulphan is quantitated by analysis on a GCMS.

Results
From the small sample of patients tested so far results will be present-
ed regarding the AUC concentration determined vs the target con-
centration.

Discussion
We are continuing to assess the significance of analysing busulphan for
therapeutic drug monitoring. From the sample analysed so far the
results look promising. The opportunity to set up an assay in collabo-
ration with a number of other clinical departments has been reward-
ing and may lead to further collaboration in the future.

HELLP Syndrome – Review & Case Study
Julie Cederman
Canterbury Health Laboratories

HELLP (Haemolysis with Elevated Liver Enzymes and Low Platelets) is a
severe form of pre-eclampsia occurring in 5-10% of all pregnancies. It
is the major cause of maternal and foetal morbidity and mortality.
Rapid development of oedema with a rise in blood pressure usually sig-
nals the onset of pre-eclampsia. Other characteristics may include;
abnormal liver function, hyperreflexia, headache, thrombocytopenia,
elevated lactate dehydrogenase, microangiopathic haemolysis, pro-
teinuria and hyperuricaemia.

In severe pre-eclampsia and eclampsia, thrombocytopenia and
microangiopathic haemolytic anaemia combined with seizures and
other organ dysfunction represent a disorder resembling TTP/HUS
called HELLP syndrome. Hospitalisation is indicated in patients with
HELLP, as DIC may develop. Prompt delivery is the optimal treatment.

The platelet count is usually at its lowest in the first postpar-
tum day. Platelets usually normalise within one week and blood pres-
sure is usually restored within two weeks post delivery. Renal and liver
function eventually return to normal.

This paper presents the case of a woman diagnosed with
HELLP during her first pregnancy. The patient presented at 25 weeks
gestation with epigastric pain and vomiting. She was found to have
mildly deranged liver function and hyperuricaemia but normal blood
pressure. After admission the patient began convulsing with blood
tests showing thrombocytopenia and greatly increased liver enzymes.
Her blood pressure was also raised.

After delivery of the baby, the patient remained in hospital for
several weeks. Her blood pressure, liver function and platelet count
eventually returned to normal.

Emerging Fungal & Aerobic Actinomycete Pathogens – The New
Zealand Scene
Dinah Parr
Scientific Officer
Auckland Mycology Unit & Mycology Reference Laboratory
Auckland Hospital

It is a year since the New Zealand Mycology Reference laboratory
transferred from ESR Wellington to the Mycology unit at Auckland
Hospital. During this period many interesting isolates have been
referred for identification or confirmation. These include aerobic
Actinomycetes, Dermatophytes, Saprophytic moulds and yeasts.
Following world trends, emerging systemic fungal pathogens such as
Fusarium species and Trichosporon species and an increase in
Aspergillus and Zygomycete infections have been seen. Unusual der-
matophytes are arriving with the immigrants from Africa and we have,
noted an increase in aerobic actinomycete infections. A resume of
the first year of this identification service and the work of the Mycology
Reference Laboratory will be presented.

Drug Resistant Streptococcus Pneumoniae in the Manawatu
Dr Jane Parker
Clinical Pathologist
Medlab Central

Streptococcus pneumoniae is a leading cause of otitis media, sinusitis,
pneumonia, bacteraemia and meningitis in both children and adults.
In recent times there has been a worldwide trend towards increasing
resistance to multiple antibiotics amongst pneumococci. This trend is
also being seen in New Zealand with a marked increase in both peni-
cillin resistance and cefotaxime resistance particularly in the last two
years.

This paper will look at these trends and will also examine the
emergence of a distinct clonal pattern seen amongst resistant S.pneu-
moniae isolated from Palmerston North and Christchurch.

TB Meningitis Case Study
Tim Langford
Microbiology Department
Medlab Central

An 11-month-old Tongan child presented with a 2 week history of
cough, intermittent fever, sweating, lethargy and poor feeding. A
chest X-ray on admission showed right lower lobe consolidation.
Lumbar puncture revealed evidence of meningitis.

A provisional diagnosis of Tuberculosis meningitis secondary to
pulmonary Tuberculosis was made and appropriate treatment started
immediately.

The contribution of various diagnostic and laboratory proce-
dures that enabled a definitive diagnosis is discussed.

Incidence of Arcanobacterium Haemolyticum and Haemolytic
Streptococci Isolated From Throat Swabs Over a 12 Month
Period
Louise Utiera
Microbiology Department
Medlab South
Christchurch
The isolation of Arcanobacterium haemolyticum and haemolytic Streptococci in throat swabs were evaluated over a 12-month period. 12985 throat swabs were examined for both A. haemolyticum and haemolytic Streptococci species using both a selective media and non-selective media. A. haemolyticum was isolated from 55 patients with the highest incidence occurring in 15-20 year old patients. Group A Streptococci were found to have the highest incidence occurring in 15-20 year olds. Group C Streptococci had the highest incidence in 15-20 year olds and Group G Streptococci in 20-25 year olds. Seasonal variations were also observed for each of the organisms isolated.

Bott Fly Infection
Jan Deroles-Main
Medlab Central

A tramping holiday in Bolivia turned into a rapid trip back to New Zealand for a 36-year-old woman after she developed a swinging fever and a painful infected leg.

Examination of several punctate wounds in the leg revealed small white larvae, which were sent to the lab for identification. This provided a rare opportunity to study a parasite not normally encountered in this country.

Homocysteine: A New Marker of Vascular Risk
Dr Peter M George
Clinical Biochemistry Unit
Canterbury Health Laboratories

Hyperhomocysteinaemia is a recently identified important risk factor for atherosclerotic vascular disease and thrombosis. This presentation reviews homocysteine metabolism, causes of hyperhomocysteinaemia, its pathophysiology and epidemiology. Screening for raised homocysteine levels should be considered for all patients at high risk of vascular and thromboembolic disorders. It appears that approximately 10 per cent of coronary artery disease within the population is attributable to homocysteine. Although prospective clinical trial data is not yet available, treatment should be considered to patients with raised levels. This is conveniently administered as a multi-vitamin supplement containing folic acid combined with vitamin B6 and B12. Until prospective data is available, vitamin supplementation provides a safe, evidence-based approach to the management of cardiac risk to patients with hyperhomocysteinaemia.

Dengue Virus Testing at Auckland Healthcare Laboratory Services – The First Two Years
Paul Austin
Laboratory Technologist
Department of Virology & Immunology
Auckland Healthcare Services Ltd

A retrospective study revealed 478 patients had been tested for Dengue virus serology at the Virus/Immunology Laboratory, Auckland Healthcare Services between April 1996 (commencement of testing) and March 1998. Patients’ serological status was differentiated into Past infection, Primary infection and Secondary infection by using commercial IgG and IgM ELSAs in parallel.

One quarter of patients tested had current Dengue infection with secondary cases twice as prevalent as primary cases. All laboratory cases were either imported infections or referred from patients hospitalised overseas.

Clinical cases of both primary and secondary infection followed up from Auckland Hospital admitted patients showed agreement with serological findings.

Highest numbers of patients tested in both sexes were aged between 16 and 47 years. For males, the rate of Dengue infection remained constant irrespective of age, whereas for females there was a notable decline in infection rate with increasing age. An interesting feature was the high rate of secondary as compared with primary infection in males aged <15 years. For females in this age group primary and secondary infection rates were equal.

The majority of requests for Dengue serology came via the local GP network although other significant contributors were from local (Auckland) hospitals, Christchurch and Pacific Islands.

By offering the service we have been able to:

(a) provide a rapid turnover time of reporting to Pacific Islands during epidemic outbreaks,
(b) notify Public Health officials of Dengue infections in community based patients, and
(c) allow hospital based clinicians to confirm or exclude Dengue infection in seriously ill hospitalised patients.

Confirmation of Immunoassay Drug Screening
Grant Moore
Toxicology Section
Canterbury Health Laboratories

Introduction
With the current use of immunoassay techniques for detection of drugs of abuse there is a need to confirm the positive results especially in situations where punitive action may be taken against the person supplying the urine sample. This may be in a workplace, school or drug treatment clinic.

Methods
On setting up our laboratory to perform workplace testing we decided to work to the Australian Standard for recommended practice for the collection, detection and quantitation of drugs of abuse in urine (AS 4308-1995). Immunoassay screening for drugs of abuse is carried out and all samples testing positive are submitted for confirmation by GCMS. Specific criteria are required for confirming the presence of drugs of abuse and, unless the analysis falls within these criteria, the sample is reported as a negative.

Discussion
We are currently performing confirmation of positive opiates, cannabinoids, methadones and cocaines using mass spectral analysis to a standard that will stand up to legal scrutiny. There are also opportunities for interpretation of metabolite patterns especially with regard to the opiates. Some of the issues regarding interpretation of the results will be discussed.

Tryptase Analysis
John Scott
Laboratory Technologist
Department of Virology & Immunology
Auckland Healthcare Services Ltd

Tryptase is one of the mediators released from Mast cells during anaphylactic reactions and a significantly raised level is a good indication that anaphylaxis has indeed occurred.

Histamine has also been used as an indicator of anaphylaxis but has proved difficult to work with. Histamine levels are elevated for an extremely short time, 15 to 30 minutes, and are very unstable on storage. Tryptase is elevated up to six hours and is remarkably stable on storage enabling much more reliable results to be obtained.

Tryptase levels are normally performed on blood samples and the time the specimen is taken has a significant effect on the level of tryptase found. Tryptase levels start to rise immediately after the allergenic challenge and are found at significantly high levels 30 minutes
later. Maximum levels are found between one and two hours post challenge with levels gradually reducing over the next few hours reaching baseline levels after six hours or greater. If one specimen is taken it is essential to take it between 30 minutes and two hours to ensure that maximal levels are detected. It is not always known exactly when a reaction starts and we recommend that three specimens be taken to ensure that peak levels are not missed. The recommended timing is 0-30 minutes, 1-2 hours and greater than six hours - preferably 12 hours. A typical graph is produced in anaphylactic reactions.

We have found that levels less than 5ug/L exclude allergic involvement (assuming correct timing of specimens). 5-15ug/L is a grey area where the third specimen system is helpful for interpretation and greater than 15ug/L supports the diagnosis of anaphylaxis.

Evaluation Of the LCR Assay to Detect Chlamydia Trachomatis in Urine Specimens from Men and Women

Michael Brokenshire
Department of Clinical Microbiology Auckland Hospital

Objectives
To evaluate the Abbott ligase chain reaction (LCR) assay to detect Chlamydia trachomatis in urine samples from men and women, and compare its performance with the current routine screening test, the SYVA!Behring EIA, in two separate studies.

Methods
Patients from the Auckland Sexual Health Clinics were included in the studies. A total of 397 urine samples from males were tested by LCR and EIA. A total of 374 urine samples from females were tested by LCR and combined cervical/urethral swabs by EIA. Grey zones were used in the EIA method and in the LCR assay for the male study.

Results
The overall prevalence of Chlamydia trachomatis was 8.9% (69/771). For males, LCR detected 34 and EIA 22 of the total 34 positives, giving sensitivities of 100% and 65%, respectively. For females, LCR detected 32 and EIA 30 of the total 35 positives giving sensitivities of 91% and 86%, respectively. In the male study there were five LCR grey-zone samples, each of which were confirmed positive, representing an increase of 9.7% (3/31) more positive over and above those that were positive only above the cut-off.

Conclusion
These studies have shown that the LCR detects significantly more Chlamydia infection in urine samples from men but not for women. The latter highlights the importance of including a urethral sample when screening for Chlamydia in women. The addition of a LCR grey-zone increases the sensitivity of the assay.

Note: Reference will be made during the presentation to other published LCR studies on urine samples.

References:

A six-page Summary of this presentation can be obtained from the presenter by emailing him upon: MikeBroke@ahsl.co.nz

Auto-Lipa – An Automated System for HLA Typing

Holly Perry
Auckland Regional Blood Centre

Introduction

Auto-Lipa is an automated system for reverse hybridisation of PCR products to oligonucleotide probes immobilised on membrane strips. One Auto-Lipa application is HLA Class II typing.

Method
During November and December 1997, Auckland Regional Blood Centre trialled the Auto-Lipa system to HLA DR type all clinical samples for transplantation purposes. During the trial, 69 samples were run in parallel, by Auto-Lipa and by the existing method; a multi primer PCR-SSP kit.

Results
The Auto-Lipa results showed 100% concordance with the PCR-SSP results. The repeat rate on Auto-Lipa was 0%, in comparison with a 12% repeat rate with PCR-SSP. Auto-Lipa gave 2-3 times reduction in the labour content of the test.

Summary
Auckland Regional Blood Centre changed to Auto-Lipa in January 1998 for all clinical Class II typing. It is hoped to use Auto-Lipa for Class I typing as soon as kits become available.

Reference
Inno-Lipa DRB key and Auto-Lipa Typing manuals; InnogeneticsNV, Canadastraat 21 - Haven 1009, B2070, ZWIJNDBEELG.

Detection and Identification of Mycobacterium Tuberculosis Directly from Specimens by Ligase Chain Reaction

Leo Mc Knight
Microbiology Laboratory
Wellington Public Hospital

Introduction
Specimens received for the diagnosis of mycobacterial infections were tested by a ligase chain reaction (LCR)-based assay, acid fast stain and culture techniques.

Methods
Results from the LCR assay (Abbott LCx Mycobacterium tuberculosis (MTB) Assay) were compared to results from standard cultures held for 6 weeks.

Results and Discussion
One hundred and eight-seven specimens from 129 patients were tested. Twenty-three (12%) of the specimens were culture positive for M. tuberculosis, and of these 19 were smear positive (83%). LCR sensitivities and specificities compared to culture were both 96%. LCR sensitivity was 100% for smear positive specimens and 75% for smear negatives.

The single LCR-negative, culture positive specimen contained low concentrations of M. tuberculosis as only three colony-forming units grew on the solid media.

Of the six LCR-positive, culture negative specimens, four were from patients on antibiotic therapy for tuberculosis, the fifth specimen was from a patient who had previously been treated for tuberculosis disease. The sixth LCR-positive culture negative specimen was a smear positive lymph node, from a patient with suspected tuberculous lymphadenitis. With these considered culture misses final LCR sensitivity, specificity, positive predictive value and negative predictive value were 97, 100, 100, and 99%, respectively.

The same performance values for acid-fast smear positivity were 76, 95, 73, and 96%, respectively. After routine specimen processing, the Abbott LCx M TB can be completed in 6 hours. If urgently required, results are usually available the day following receipt of specimen.

Viral Load Quantitation Using Branch DNA

Brian Schroeder
Auckland Healthcare Laboratory Services
The first case of AIDS in New Zealand was diagnosed in 1983. As of 31 March 1998 the total AIDS notifications amounted to 649 with a cumulative incidence rate of 18.9 per 100,000. The total number of individuals found to be HIV antibody positive in the same period is 1260. With the advent of effective antiretroviral therapy a means to monitor patient response became essential. Since November 1996 the Auckland Hospital virology laboratory has offered HIV viral load testing using the Chiron Quantiplex branched DNA assay. Over 400 patients have been monitored regularly. Some of the patients acquired their HIV in Asia or Africa introducing genetic subtype variants into New Zealand. Data will be presented showing the relevance of HIV viral load quantitation and HIV-1 subtype variation.

The Value of Information – Why a Standardised Costing Model? Walter Wilson Executive Officer Blood Transfusion Trust

Over the period 1995 to 1998 the Blood Transfusion Trust developed a model to establish the costs of the operational activities of the New Zealand blood service and to report the individual costs of the blood products produced by each Blood Service provider using a standardised model. The paper discusses the principles used by Deloitte Touche Tohmatsu (consultants to the Trust), to develop the model, the processes followed and potential uses for the information reported from the data supplied by the blood service providers.

Donation Screening for Antibody to Yersinia Enterocolitica Among Manawatu Blood Donors Chris Kendrick Lecturer, Institute of Veterinary Animal & Biomedical Science Massey University C.J.Kendrick@massey.ac.nz

Since the early 1990s an increasing incidence of the bacterial infection of blood components has been reported internationally. Most cases followed the transfusion of platelet concentrates but a greater than expected incidence has been noted following the transfusion of red cell concentrates (RCC). In about 90% of the cases reported the causative organism was Yersinia enterocolitica a gram negative psychrophil, perhaps better known as a gastrointestinal pathogen. The reason(s) for the increased incidence are not altogether clear although it has been suggested that the now routine practice of plasma depletion during component preparation may be symptomatic. NZ has some of the highest recorded rates of GIT infection caused by organisms such as Campylobacter, Salmonella and Yersinia in the developed world.

Between 1991-1995, unit infection with Y.enterocolitica led to transfusion related endotoxic shock and life threatening complications in recipients of eight red cell packs in NZ. Since 1995 a further two units were discovered to be heavily infected luckily before they were transfused. High rates of unit infection in NZ have been reported in the scientific literature (Theakston et al.) The prevention of further cases which still represent the single greatest threat to recipients of blood in NZ is not easy. Steps adopted to prevent more cases have impacted adversely upon donor recruitment and unit utilisation and for the moment appear to have reduced the incidence.

Preliminary studies into the immune response in those giving blood that later showed to be heavily infected reveal the potential for screening donor sera for current infection with Y.enterocolitica. A new Elisa assay, has been developed at Massey University and is currently under trial in the Blood Centre at Mid Central Health Ltd. The assay has been designed to detect current/recent infection with Yenterocolitica and is being trialed to establish its usefulness as a means of screening donated blood to eliminate further transfusion reactions caused by Yenterocolitica. Performance of the assay against donor sera, sera from confirmed cases of unit infection, and the sera from patients with culture confirmed Yenterocolitica infection are presented.

Clots and Contraception
Dr Paul Harper Consultant Haematologist Mid Central Health Ltd.
Palmerston North

The oral contraceptive was introduced into clinical use in the late 1950s but it was not until 1961 that the first case report of venous thrombosis associated with the pill was reported. Over the course of the next few years it became apparent that oestrogen-containing oral contraceptives were associated with approximately a ten-fold increase in the risk of venous thrombosis in otherwise healthy women. This observation led to the proposal that changes in coagulation factors could be responsible. In general ethinyl estradiol, the most commonly used oestrogen, has a small effect on individual clotting factors, but overall the combined coagulation changes are unfavourable. The most important are an increase in factors VII, X, prothrombin and fibrinogen, with a reduction in antithrombin and protein S. These adverse effects are to some extent balanced by an increase in fibrinolysis. Although much uncertainty remains about the precise cause of thrombosis, the oestrogen content of the pill was thought to be the significant factor. Early contraceptives contained 100µg of oestrogen, whereas present agents have between 30 and 35µg. The reduction in oestrogen content has considerably reduced the incidence of thrombosis.

Recent studies have shown that second generation combined oral contraceptives are associated with a four-fold increased risk of thrombosis. The risk is even higher in women who smoke or those with obesity or hypertension. Women particularly at risk are those with an associated thrombophilia. Until recently this accounted for a small number of cases but since the identification of the factor V Leiden mutation management has become more complex. Approximately 4% of Europeans are heterozygous for this mutation and their risk of thrombosis is eight times higher than normal. Women with this mutation who take the pill have a risk 35 times normal. Although this is a high risk in real terms the incidence of thrombosis still remains very low and raises the question should all women be screened for factor V Leiden before starting the pill? In New Zealand approximately 150,000 women take the pill. Each year twenty-two thromboses are associated with the pill and one fatality occurs every two years. If all women were screened and all women with factor V Leiden were refused the pill this would prevent eight thromboses each year and one fatality every six years.

The Proposed World Health Organisation (WHO) Classification of Leukaemia and Lymphoma
Joy Monteath
Canterbury Health Laboratories

The classification of leukaemia and lymphoma was initially made on cytological and histological appearances supplemented by cytochemistry. In 1976, acute myeloid and lymphoid leukaemias were defined in greater detail by the French American and British (FAB) Group and revised in 1985. Since the 1960s several attempts to classify lymphomas have been made by many different groups, and include the Rappaport (1966) and Kiel (1984) Classifications, and the Working Formulation
(1982). The Revised European and American Lymphoma (REAL) Classification (1994) aimed to include biological information derived from the new techniques of phenotyping, genotyping and cytogenetic analyses, combined with clinical data.

In 1996, the WHO sought to broaden the consensus findings of the REAL Classification, and to include updated classifications of myeloid and lymphoid leukaemias to take account of the many advances in cell biology. Committees of European, British and American haematopathologists and clinicians were established to achieve this objective and the proposed WHO Classification of Neoplastic Diseases of Haemopoietic and Lymphoid Tissues was introduced by the European School of Haematology in London April 1998. It is scheduled to be published in 1999.

A description of this new classification will be given with particular reference to the way in which the laboratory techniques of phenotyping, genotyping, and cytogenetic analyses can aid in the diagnosis of these diseases.

Overview of Trauma Management
Dr Peter Hicks
Director of Intensive Care
Mid Central Health Ltd.
Palmerston North

If trauma was a disease it would be an epidemic. Minor trauma injuries occur to us all and are one of the commonest reasons for hospital admissions. Major trauma injuries are very uncommon but are the commonest cause of death for people under 40 years of age.

Trauma management comes in two parts: Firstly, you develop systems that coordinate the pre-hospital emergency services so that injured people get early, skilled care and rapid transport to an appropriate hospital. Secondly you develop hospital guidelines to provide good patient care.

Head injuries are a particular problem. Major head injuries cause almost all the in-hospital trauma deaths while minor concussions are now being recognised as causing significant impairments for up to six weeks.

Ultimately injury prevention is the best management we have.

Future Trends in Red Cell Testing
Jeanette Corley
Production manager
CSL Biosciences
Melbourne

This century we have seen the development of a wide range of testing systems, the purpose of which is to assist in the selection of blood for transfusion that will have acceptable survival when transfused and will not cause harm to the recipient.

We will look at the ‘Paradigm Shift’ that is occurring in pre-transfusion testing and discuss some of the future potential technologies that we may encounter in the testing laboratory as we head into the millennium.

Blood Processing Quality Control. Makes an Investment
Margaret Dickinson
NZ Blood Service Northern
Auckland

Introduction
The NZ Standards for the collection, processing, and distribution of Blood and Blood Products contains the specifications for a range of parameters. Considerable effort is expended taking and analysing samples during blood processing. The resultant data can be faithfully recorded for presentation to curious auditors annually or it can become a gilt edged investment.

Statistical process control (SPC) is the key to changing energy expended into an investment.

Methods
SPC methods range from plotting graph paper charts to sophisticated software. Utilising data obtained during routine processing in our blood centre, the basics of x-bar, sigma charts are demonstrated. Templates for these charts have been developed using Microsoft Excel™. Processing problems are identified, random or systematic causes determined and corrective action instituted.

Results
The in house charts have proven very useful, however a reasonable level of competency with Microsoft Excel™ charts and statistical experience are required to get maximum information from the available data. There is now a range of sophisticated commercial packages available. Ql Analyst 3.5 Demonstration Version downloaded from the Internet is one such tool.

While a suitably trained technologist or statistician is required to set up the formats, the software can be used in the routine processing department with the option for download of data from analytical instrumentation eg balances, cell counters. There is easy recognition of sample requirements, data entry requires operator identification, alarms alert operators to non-conformances, and corrective actions are recorded. In line statistical process control can become a reality.

Discussion
The Ql Analyst 3.5 software demonstrated is only one of a number of commercial SPC packages available. The software's presentation on the Internet, successful download, and quality of the demonstration programme, suggest it would be an excellent investment for the New Zealand Blood Service.

Quality Control in the Clinical Laboratory
Elizabeth Bonagara MHP, MT (ASCP)
Bio-Rad Laboratories Australia

As an introduction, the first half of this session will include presentations on ‘The Basic Concepts of Quality Control’ and ‘The Benefits of Participating in an External QC Program’. All attendees will receive informational literature and are encouraged to participate in a summary round table discussion.

The second half of the session will include an ‘Introduction to Bio-Rad Laboratories’ and a Workshop on ‘Bio-Rad’s UNITY-PC Quality Control Program’—Beginner and Advanced Training. All current and future UNITY participants are encouraged to attend, as many practical and advanced uses of the program will be demonstrated and discussed.

Which Analyser?
Katherine Denton
Core Haematology
Canterbury Health Laboratories

Three new generation haematology analysers, the Abbott CellDyn 4000, the Coulter GenS and the Bayer ADVIA 120 have been evaluated at Canterbury Health Laboratories between November 1997 and June 1998. The analysers have been assessed for their suitability for use in our Core Haematology Laboratory. The analyser of choice will replace our ageing Coulter STKS analysers. The aim is to find which analyser would best meet our requirements of:

- speed
- reliability with a reduction in regular maintenance and troubleshooting
reduction in false flagging and better definition of cells which
would result in reduction in film reviews
automated reticulocyte analysis
ease of use particularly for non haematology qualified opera-
tors
greater linearity
acceptable precision and accuracy
performance of controls and quality control package
option of sidemaker stainer is also considered

Each analyser will be discussed as regards the above features
and any other additional features that were of interest or benefit.
Experiments were performed to measure linearity and stability
of results. Within batch and between batch precision checks were
performed. Comparisons with the Coulter STKS were done with all pa-
rameters. Differences were also compared to the manual differential.
Reticulocytes were compared to our current manual method, as was
nucleated RBC counting for the CellDyn 4000 only. Data on flagging
was analysed using truth tables. Specificity, Sensitivity, Predictive values
and Efficiency were calculated. Throughput was determined and data
on maintenance and troubleshooting was recorded.

Results of the above analyses are presented as well as specific
examples of interesting and abnormal samples. A summary of the per-
formance of each analyser is presented along with potential advan-
tages and disadvantages of the system.

Katherine Denton, Lesley Newton, Linda Williams
Core Haematology, Canterbury Health Laboratories.

Near Patient Testing: A Solution for the Isolated Country
Hospital
Lorraine Craighead & Dr Martin Watts
Balclutha Hospital

Healthlab Otago has utilised Near Patient Testing in Balclutha Hospital
to provide a unique solution to the delivery of timely, accurate, and
economic laboratory services to a small isolated rural hospital.

Near Patient Testing is laboratory testing occurring outside the
physical confines of the laboratory, but under the control of the cen-
tral laboratory. Precalibrated or autocalibrating instruments along with
computerised communications with the central laboratory means that
operators do not require specialised laboratory training. The introduc-
tion of Near Patient Testing requires careful thought, training, supervi-
sion, and above all communication to succeed. The laboratory has to be
committed to making it work.

Instrumentation consists of a Coulter MD18 for Haematology,
an Easylyte and Reflotron for Biochemistry, and a Statpak for blood
gases. These are all compact robust instruments with which very little
can go wrong. Instruments, which produce printouts of results, are essen-
tial. The instruments were initially introduced one at a time and
procedures were carefully altered to suit the parties involved.
Consultation and communication with all ward staff was paramount
to making this exercise a success.

The doctors have frequent refresher courses, and their work is
closely monitored. The laboratory complies with International
Accreditation standards. Responsibility for the accuracy and documen-
tation in medical and laboratory records rests firmly with the laborato-
ry. When near patient testing volume increases, costs rise substanci-
ally but when volume falls costs fall just as drastically. The substitution of
variable costs is a very economic method of dealing with variable
volumes. The future of near patient testing may involve the training of
nurses to operate the instruments and off site supervision by the con-
trolling laboratory.

Misadventure and the Liability of Healthcare providers
Graham Rossiter

Senior Lecturer in Healthcare Law
Department of Management Systems
Massey University

The liability of health care providers in the broadest sense may arise
under several headings, namely with reference to:
1. a civil action in the ordinary coin.
2. a complaint under the Health, and Disability Commissioner: Act
1994 with the possibility of proceedings before the
Complaints Review Tribunal,
3. a complaint to the relevant professional body.
4. with respect to the Health Information Privacy Code, a com-
plaint to the Privacy Commissioner and
5. if death or injury results from negligent error on the part of the
provider, a criminal prosecution.

It is well known that successive accident compensation legisla-
tion (currently the Accident Rehabilitation and Compensation
Insurance Act 1992) restricts the remedies available to a health con-
sumer against a provider.

Basically, where there is Accover® under the accident compen-
sation legislation, there is no right to sue for damages, except
Aexemplary® or punitive damages.

The accident compensation legislation of 1992 has narrowed
the definition of Accover® under that Act and consequently enlarged
the civil liability of the health provider.

Recent Developments in Accreditation of Medical Testing
Laboratories
Grahani Walker
Programme Manager Medical Testing and Radiology
International Accreditation N2

Internationally, the accreditation of laboratories, including medical
testing laboratories, is an ever changing activity. New Zealand is not
immune to international trends, and thus to remain in tune with the
international accreditation scene, the International Accreditation New
Zealand (IANZ) process for accrediting medical testing laboratories in
New Zealand, must also change.

Significant among recent changes is the separation of the
commercial ISO 9000 certification activities of Telarc Ltd. From the
government appointed ISO Guide 25 accreditation activities of the newly
named International Accreditation New Zealand. The reasons for, and
the ramifications of this change, are discussed.

A summary of the current status of the IANZ medical labora-
tory accreditation programme is presented, and recent developments in
other IANZ programmes relevant to the health care industry are also
summarised.

Increasing consumer awareness of quality issues, commercial
competition, organisations risk management, funding authority or
purchaser requirements and other related pressures, are now com-
monly applied to many medical testing laboratories, and thus for many
laboratories the attainment and continued maintenance of IANZ
 accreditation carries a new and more urgent meaning.

The IANZ team, once invited to medical laboratories on a vol-
untary basis every two or three years, now visits at least annually and
effectively on a mandatory basis. The changes, both positive and neg-
ative, brought about by the more frequent visits and the somewhat
less flexible arrangements currently implemented by IANZ, are dis-
cussed in detail.

A summary of the cost structure applied to IANZ medical lab-
aboratory programme is presented and discussed. Also discussed are
some new initiatives that are hoped will add value to the IANZ pro-
gramme, while not contravening the requirements of the internation-
al mutual recognition agreements that IANZ operational procedures
are now required conform to.

The presentation will include the use of typical laboratory scenarios and examples to illustrate the topics discussed.

Hospital & Community Laboratories – Don’t Let This One Get Away!
Dr Cynric Temple-Camp
Pathologist
Medlab Central

The international trend in laboratory medicine is the rationalisation of services, which has invariably been financially driven. In all OECD countries the amount of laboratory testing has increased and this has been driven by an ageing population, increased monitoring requirements for a variety of diseases and medications, the increasing sophistication of available tests, public perception of current practice as well as the more obvious medical legal issues. In most countries the demand for laboratory services has grown at a rate faster than other medical services.

A variety of methods have been used to reduce the growth in cost in laboratory testing. The fusion of hospital based laboratory practice together with general practice community based practice is one possible method which has been tried in Calgary as well as at a number of sites within New Zealand. This discussion looks at the different requirements of the hospital versus community based testing and examines the issues of possible economies of scale, the problems both seen and unforeseen as well as an analysis of whether there is a New Zealand based model for the integration of the different services.

Occupational Safety and Health: A Health Perspective
Dr Ian Laird
Department of Human Resource Management
Massey University

Introduction
This paper reviews historical aspects of medical laboratory practice in New Zealand, particularly in relation to health hazards, and provides preliminary results of a cross-sectional survey of perceived hazards and work-related symptoms in medical laboratory staff in the Central region of NZ.

Method
Twenty-five private and public medical laboratories were identified from the NZIMLS database and all staff (including administration staff) were encouraged to complete a self administered questionnaire.

Results/Discussion
A range of physical, chemical, biological health hazards was identified, in addition to musculo-skeletal discomfort and psychosocial stressors. The adequacy of the ergonomic aspects of the laboratory work environment was also assessed by respondents. The use of personal protective equipment was found to be very frequent and staff appear well informed of consequences of exposure. A range of work related symptoms due to aerosols and air quality of the laboratory were identified. The preliminary results suggest that medical laboratory staff are exposed to a wide range of health hazards, but that the prevalence of work related symptoms is low.

Needle Stick Injuries, Risks, Costs and Prevention
Terry R Grimmond
Managing Director
The Daniels Corporation NZ
PO Box 4016
Hamilton.
Service@daniels.co.nz

Needlestick injury (NSI) among Health Care Workers (HCW) peaked throughout the world in the mid-eighties and has declined somewhat since then. The nature of NSI (when, how and why) has also changed. The reasons for NSI decline are:
- more effort is used to eliminate sharps injuries,
- safer sharps containers.

The reasons for this increased diligence were the new occupational Health & Safety Acts brought about by a single phenomenon – the risk of contracting AIDS.

In the USA the peak of NSI was calculated at over 1 million per year and is now 800,000. Extrapolating, NZ still suffers an estimated 11,000 NSI annually. Only half are reported.

The cost of investigating NSI has risen sevenfold and is estimated currently at $150 per episode, however one acquisition of HBV may equate to 42% of all NSI costs over a five-year period and even NSI from sterile needles can have disastrous consequences.

The risk of NSI averages 0.2 NSI per HCW per year. Laboratory personnel are among the top three groups at risk. The risk of HIV acquisition following NSI ranges from 1 in 200,000 on a general ward to 1 in 300 on an AIDS ward. The causes of NSI are: insufficient NSI education; insufficient containers; poor container design; and increased workload. NSI prevention is brought about by: less use of sharps; NSI awareness; easy access to sharps containers; user-friendly sharps containers; not overfilling sharps containers.

Are Clinical Laboratories in NZ Able to Detect Antibiotic-Resistant Enterococci?
Dr Maggie Brett
Antibiotic Reference Laboratory
Institute of Environmental Science and Research
Porirua

Introduction
Antimicrobial-resistant enterococci, particularly vancomycin-resistant enterococci, have emerged as a major public health problem in many countries. Such strains pose therapeutic dilemmas and clinical laboratories need to be able to detect resistance accurately. Ten laboratories in New Zealand participated in an international multicentre study for the detection of antimicrobial-resistant Enterococcus spp. initiated by the Centers for Disease Control and Prevention, Atlanta, and co-ordinated by ESR.

Methods
ESR distributed five coded enterococcal isolates to the participating laboratories, together with a standard protocol. The laboratories were requested to identify the isolates and to test the isolates for resistance to vancomycin and penicillin or ampicillin using the antimicrobial susceptibility testing method routinely used in their laboratory.

Results
All laboratories correctly detected an E. faecium isolate with high-level vancomycin resistance and the majority of laboratories detected an E. faecium isolate with a vancomycin MIC of 64-256 μg/L. However, an E. faecalis isolate with a vancomycin MIC of 16-64 μg/L was correctly detected by only 40% of the laboratories. Ampicillin/penicillin resistance was correctly detected by the majority of laboratories.

Discussion
The results indicate that New Zealand laboratories may have problems in detecting low-to-moderate-level vancomycin resistance but not high-level vancomycin resistance or ampicillin/penicillin resistance in enterococci.

Haematology’s Liver Transplant Experience
Glen Devenie
Department of Haematology
Auckland Healthcare Laboratory Services
The use of the New Zealand Liver Transplant Unit in the middle of 1997. The first operations were expected to start in February 1998. The haematology laboratory has had to make an on-call roster to cope with the intensive nature of the operation. The person who is on call is phoned to come into the laboratory when a transplant is happening. That person is responsible for registering, processing and phoning the coagulation results to theatre. In acute admissions for potential liver recipients Factor V assays are performed urgently.

The first liver transplant in New Zealand was performed at Auckland Hospital on February 20th 1998. The patient was an acute admission with liver failure presenting with an INR of 15.7, prothrombin time of 180 seconds, APTT of 115 seconds and a fibrinogen of 0.3 g/L. There have been four transplants to date and all have been successful.

The operation has five phases Preparation, Induction to hepatectomy, Anhepatic phase, Reperfusion, Post reperfusion to Intensive care. Haemoglobin, platelets, prothrombin time and fibrinogen are performed regularly throughout the operation. The anaesthetist in the theatre tests and blood gases and Trombelastographs (TEG). During the operation the prothrombin ratio increases and fibrinogen drops. When the new liver is reperfused the prothrombin ratio drops and fibrinogen rises autonomously. The new liver removes, lactate, glucose and potassium from the blood, corrects acidosis and releases coagulation factors, which corrects the coagulopathy.

**Bibliography**


**Disc Diffusion Test for Detection of Penicillin Resistance in Streptococcus Pneumoniae**

Sarah Hopkirk

**Antibiotic Reference Laboratory**

**Institute of Environmental Science and Research**

**Introduction**

The use of a 1 μg oxacillin disc as a screening test to detect penicillin resistance in *Streptococcus pneumoniae* as recommended by NCCLS is a highly sensitive but not specific test. This study compares the use of the 2 μg penicillin disc and the 1 μg oxacillin disc for differentiating between penicillin-resistant, penicillin-intermediate and penicillin-susceptible pneumococci.

**Methods**

A total of 419 clinical pneumococcal isolates were tested. The penicillin MICs were determined using E-tests and disc diffusion tests with 2 μg penicillin and 1 μg oxacillin discs were performed in parallel following NCCLS guidelines.

**Results**

Of the 419 isolates tested, 131 were penicillin-resistant (MIC ≥2.0 mg/L), 136 were penicillin intermediate (MIC 0.12 - 1.0 mg/L) and 152 were penicillin-susceptible (MIC ≤0.06 mg/L). Using the NCCLS recommended breakpoint of > 20mm for penicillin resistance, all the penicillin-resistant and penicillin-intermediate isolates were correctly classified by the 1 μg oxacillin disc. For the 2 μg penicillin disc, zone diameters of ≤ 12mm were interpreted as resistant, 13-25 mm as intermediate and ≥ 26mm as susceptible. Of the 419 isolates, 125/131 (95.4%) were correctly classified as penicillin-resistant; 78/136 (57.4%) as penicillin-intermediate and 150/152 (98.7%) as penicillin-susceptible by the 2 μg penicillin disc. There were no very major (false susceptible) or major (false resistance) errors but there were 66 (15.8%) minor errors with the 2 μg penicillin disc, mostly among the penicillin-intermediate isolates.

**Conclusion**

The 1 μg oxacillin disc was very sensitive and detected all the penicillin-resistant and intermediate isolates. The 2 μg penicillin disc correctly detected the majority of the penicillin-resistant isolates but did not detect all of the penicillin-intermediate isolates.

**Nuclisens-NASBA (Nucleic Acid Sequence Based Amplification)**

**John Peake**

**Pharmaco (NZ) Ltd**

The Nuclisens system is a Molecular Diagnostic tool which is based on the synergy of three core technologies:

- Nucleic acid extraction
- Nucleic Acid sequence Based Amplification
- Electro Chemiluminescent Detection

Each are integrated into an all-in-one system, targeted at the sensitive and specific detection of nucleic acid sequences.

Nuclisens has a modular system design. Automated nucleic acid extractions can be performed on the Nuclisens Extractor, isothermal amplification relying on the simultaneous activity of 3 enzymes and automated detection is performed on the ECL (Electrochemical luminescence) reader.

Currently available tests on Nuclisens technology can however be used generically for any analyte utilising the basic (to be released by the end of 1998).

**All Psyched Up and Nowhere to Go – Our Values Help Motivate Us.**

**Mike Henderson**

**Director of Research & Development**

**True North Ltd**

(1) Values – a definition

“Our Values are what motivate us!” An explanation of how they impact on our deep structure.

(2) Cluster core

Past, present, future.

Means and ends.

(3) The benefits of alignment – discover how living your values as an Individual and Organisation has numerous benefits.

**A Comparison of Ligase Chain Reaction and Enzyme Immunoassay for the Diagnosis of Urogenital Chlamydia Trachomatis**

**Collette Bromhead**

**Medical Laboratory – Wellington**

**89 Courtenay Place**

**Wellington**

**Introduction**

Chlamydiae are non-motile, gram negative bacterial pathogens. *Chlamydia trachomatis* is one of three chlamydial species known to be major aetiological agents of urogenital infections, disease in neonates born of infected mothers and complications including infertility. Traditional laboratory diagnosis has consisted of either cell culture, enzyme immunoassay (EIA), or direct fluorescence antibody stains (DFA). Studies have found test sensitivity of 78% and 65% respective­ly from EIA and cell culture while nucleic acid amplification tests such as Ligase Chain Reaction (LCR) have been found to offer sensitivity of 96%. The ability to theoretically detect a single gene copy means that nucleic acid amplification tests overcome the specimen inadequacy problem that affects methods such as EIA and culture.
Methods
We have recently changed our routine Chlamydia test from an EIA (Syva) with DFA confirmation to the Abbot LCx system (LCR). Using data taken from laboratory worksheets during two matched time periods in 1997 (EIA) and 1998 (LCR), we have surveyed a total of 15,273 swabs and urines (7644 tested by LCR and 7629 tested by EIA) and quantified the difference in detection rates between the two assays, seen in this laboratory.

Results
Our results show a 72% overall increase in the number of positive specimens when testing by LCR. This result may be skewed by an increase in the amount of positive urines tested (1020% increase for males, and 880% increase for females). However, when only cervical swabs are compared, the increase in positivity is calculated to be 51.7% and we believe this is probably an underestimate due to the practice of pooling cervical and urethral swabs for EIA, thus increasing the possibility of an EIA positive, while LCR is conducted on single cervical swabs.

Discussion
The statistics we have collated show a marked increase in the sensitivity of LCR over EIA for the detection of Chlamydia trachomatis. Our results imply that between 1 and 1.4 out of every two positive specimens were being missed by EIA detection. Whilst our study is not directly comparing single specimens tested by both methods, we believe our sample size compensates for this inadequacy and the results are a good approximation for the population of specimens being tested.

In conclusion, we consider the increase in sensitivity and specificity of LCR coupled with the ability to accurately test less invasive specimens has greatly improved our ability to diagnose Chlamydia trachomatis in genitourinary specimens.

Bio-Rad Variant β-thalassaemia Short Program. Can it be Used to Detect α-thalassaemia?
Soakimi Po'uhila
Mandy Wood
Barbara Walton
Haematology Department
Greenlane and National Women's Hospital
Auckland

Introduction
Bio-Rad Variant is an automated HPLC system for analysing haemoglobin. We use β-thalassaemia short program in our laboratory for haemoglobinopathy screening. This program is not designed to study α-thalassaemia abnormalities. We examined the usefulness of the Bio-Rad β-thalassaemia program for detecting Hb Barts hydrops foetalis.

Case
An Asian woman presented to National Women's hospital and gave birth to a baby with hydrops foetalis of unknown cause. Cord blood showed severe anaemia and in view of ethnicity we ran the sample in the HPLC analyser in addition to the alkaline haemoglobin electrophoresis.

Results
Alkaline Haemoglobin electrophoresis showed a distinct Barts band approximately 80% of the total haemoglobin with 2 faint bands representing Hb Portland and Gower. Bio-Rad variant HPLC showed 2 fast moving bands, and reported 100% HbF although there were no peaks in either HbF, HbA and HbA2 regions. The cord blood haemolysate was eluted and quantified and then analysed by HPLC method to confirm the 3 bands identified in the electrophoresis.

Conclusion
Bio-Rad variant β-thalassaemia short program is useful in detecting Barts hydrops foetalis by showing the absence of HbF and HbA and presence of the fast moving bands although it cannot identify the bands. Although the HbF was misidentified in the HPLC chromatogram, it poses no problem in the overall picture. HPLC consumables cost is around $30 per sample while it only cost $4 for the electrophoresis. However, the faster turnaround times the HPLC (30min) is important in the clinical management of the patient in instances where the cause of hydrops foetalis is unknown. HPLC is also so fully automated that it frees the Technologist for other duties. The electrophoresis method although cheaper it takes a long time (1.5hr) to process. In the interest of the patient HPLC is recommended therefore increased cost would be warranted.
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