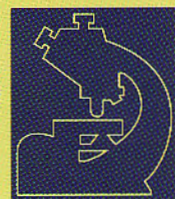


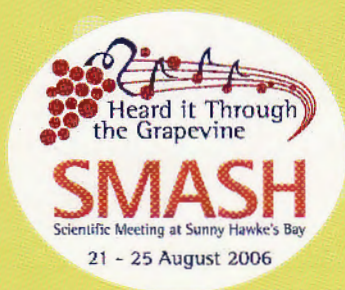
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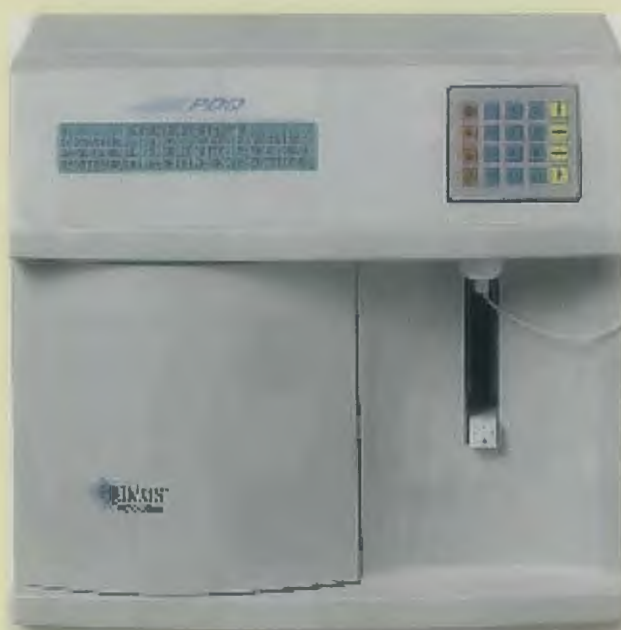
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TH Pullar Memorial Address

From the back of beyond

*Christine M Hickton, FNZIMLS
Locum Scientist, Hawkes Bay District Health Board*

Mr President, distinguished guests, colleagues and friends. On a beautiful Monday morning in mid June I wandered inside after a morning spent spraying weeds in our Makotuku vineyard to find a letter from the New Zealand Institute of Medical Laboratory Science on the dining table. Expecting confirmation of my conference registration, the words on the page appeared to make no sense at all. Instead of registration confirmation, the letter appeared to be asking ME to present the TH Pullar Memorial Address! I read it through again and that was definitely what it said! I briefly wondered about the hallucinogenic properties of the current batch of Roundup, and what would it fetch on the open market as the latest in designer party drugs! To be standing here today is a tremendous honour, and still a great surprise.

As I tried to decide what I would talk about today, I looked through several past TH Pullar Addresses and was somewhat daunted to note that most of the previous presenters were people who had come from a career context that was very different to my current one. They were people from large laboratories, people who were past Presidents or members of council of the NZIMLS, journal editors and the like. My current context is that I work as a locum staff member and support person in Wairoa, Northern Hawkes Bay, one of the smallest and more isolated laboratories in the country, so what could I possibly have to say? Ten years ago my career was on a very different path as I worked as a specialist technologist in one of the largest laboratories in the country.

I reflected that the theme of the conference, Basics and Beyond, echoed the path that my career has taken, but I have gone one step further, and my career theme has been Basics and Beyond and Back. When I finally got my head around the letter, I noted the comment that I had seen a lot of change in medical laboratory science over the years, and although that thought made me feel positively ancient, on reflection I had to concede that it was probably true.

I have been extraordinarily fortunate in my medical laboratory career to date. I entered the field of haemostasis as the science was beginning to expand. This was also about the time that the first of the inherited abnormalities leading to a thrombotic tendency was described and there was the awareness that there were possibly a number of inherited deficiencies causing thrombosis as there were inherited deficiencies leading to bleeding. Because of this timing I was to be personally involved in several new developments, both in this country and internationally. Over those years I have met some truly inspiring people and had some wonderful experiences. But my career path did not happen in isolation, there have been a number of influential mentors that I have known and learned from. I want to mention some of them today. In mentioning people by name you always run the risk of leaving somebody out, and if I have done that, I am sorry.

The most important thing that these people have in common is that they are all people who paid very careful attention to detail and who do or did, the basics well. Some of them worked in routine laboratories and some in specialised laboratories, but every level of laboratory work has a degree of basic laboratory practice, and for there to be any "beyond" in medical laboratory science, we must do the basics well, we must recognise and investigate those parts of the jigsaw that

make up a patient and their disease, that do not fit into the completed picture. In the field of haemostasis at least, many of the important new discoveries were actually made by somebody who was doing something else at the time, and I suspect that is true of many areas of medical laboratory science.

I began my training in the laboratory of Palmerston North Hospital on 12th August 1969. The late 60s were the beginning of the boom in medical laboratory science that saw the number of trainees taken on each year increase from one or two, to classes of five or six in Palmerston North, and even more in the larger centres.

We were given a good on site basic training. Lectures were held for an hour after work twice a week with first, second and third year trainees all sitting in the same lecture, the theory being that when you had completed your 3 years basic training you had sat through each lecture at least once. I have to thank Harry Hutchings, Jim Mann, Colvin Campbell and Roger Sims for the great grounding in the basics of medical laboratory work that has stood me in good stead in my career to date.

Dr Thomas Pullar, in whose memory this annual address is given, was the pathologist in Palmerston North for twenty-five years until he moved to Tauranga in 1963. He died in August 1966 almost exactly 3 years before I started work in Palmerston North.

There were two pathologists in my time, Dr Darby, a rather father-like figure who took a keen interest in all the staff, and Dr Ash Corbett, the Chemical Pathologist who scared the living daylight out of most of us. From Dr Corbett, I learned (very quickly) to keep quiet, (although I was not too good at that) keep my head down, and don't produce work that was anything less than perfect. The weeks we spent on protein bench, his particular interest, were always dreaded, but we learned to produce perfect electrophoresis strips in double quick time.

My next two years were spent at the Princess Mary Hospital for Children where I completed Certificate and Specialist level Haematology. My initial plans had been to do Certificate Haematology/ Blood Bank, as it was in those days, to then do Specialist level in Blood Bank. My main reason for that decision was that although I liked Haematology as a subject, it also included coagulation and I could never get my head around all of those clotting factors, the numbers of which appeared to me to follow no logical sequence. For some reason, the details have been lost in the passing years, at the last minute I changed my mind and settled for Haematology Specialist level instead.

On qualifying I applied for and was accepted for a position as staff technologist in the coagulation laboratory at the Auckland Blood Transfusion Service. Somewhere during the previous year I had managed to sort out all those clotting factor numbers, and had really enjoyed the 2 weeks training that I had spent there doing such specialist tests as factor VIII assays. In the mid 1970s coagulation factor assays were done only in the main centres and from memory the Auckland BTS was the only place that offered the full range.

The technologist in charge of the coagulation unit was Jim Montgomery and I worked with Jim for about 18 months before he was involved in an accident on his motorbike and sustained serious head injuries and brain damage that were to end his career. Our main role in the coagulation lab was to provide a reference coagulation service for

the Auckland hospitals, and other laboratories around the country and to quality control the blood products produced by BTS, which mainly meant assaying the Factor VIII levels in the cryoprecipitate, the only product available for treating haemophilia. Between times we made almost all of the reagents we used in the laboratory, and we worked on developing new methods. The first new method that I was involved in developing was a functional assay for measuring Antithrombin III, the first recognised inherited deficiency that could lead to a thrombotic tendency.

Towards the end of the first year of my time in the coagulation laboratory, Jim took up a Churchill Scholarship and travelled to the UK where he looked at a number of new methods and on his return we began to set these methods up in the laboratory. The two that I think had the greatest impact, and that we were able to continue developing after his accident, were the production of a standardised Thromboplastin reagent which would eventually revolutionise the way in which prothrombin time testing was performed in this country, and the assay of what was then known as Factor VIII related antigen by Laurell Rocket technique which changed the way in which Haemophilia and von Willebrands disease were diagnosed. Prior to this, the only tools available to differentiate between Haemophilia A and von Willebrands disease were a Factor VIII assay, bleeding time and the patients bleeding history and family history.

Jim was a great one for writing lists of tasks to be done as a way of mapping progress and keeping focused on the path ahead in a laboratory where you could start the day with a clear plan of attack on the methods currently being developed, and one phone call could mean that the entire day was turned up side down as a couple of you headed for one of the outlying hospitals and a patient who needed urgent investigation of their coagulation status.

A legacy from Walter Wilson's days in the coagulation laboratory, we each had a series of workbooks in which we wrote down the details of everything we did and the results of what we had found, so that all stages of method development were recorded. If there was a problem encountered or an unexpected result it was an easy task to go back over the procedure to try and establish just what had happened. Jim's lists and Walter's lead in documenting all of our steps in method development meant that as we came to accept that Jim's injuries would mean that his return to work would be some time away, if ever, the laboratory could continue to function, albeit slowly at first, along the path that he had planned for it.

Shortly after Jim's accident I attended my first Annual Scientific Meeting, which was held in Whangarei, and it was there that I met Jan Nelson for the first time. Jan had only recently moved to Auckland and was looking for a job. A couple of weeks later she was taken on as a staff technologist working with me in the coagulation laboratory. It was the beginning of a long friendship.

As Jan and I were both relatively newly qualified and somewhat orphaned by Jim's absence, the BTS research scientist, Roy Douglas, kept a bit of an eye on us and regularly reviewed our work. Roy, by his own admission knew little about the field of coagulation, and so we were required to explain in detail all of our results. If there was an unusual finding he did not expect that we would always be able to tell him what the cause was, but we were expected to have investigated the finding as fully as we were able and at least to be able to tell him what it wasn't.

From Jim Montgomery, Walter Wilson, Roy Douglas and Dr Jock Staverley, I gained a good grounding in investigative medical laboratory science, to look, to question, to explore, and try and explain.

In 1978 I moved to Christchurch and obtained a position in charge of the routine coagulation laboratory at Christchurch Hospital. At that time Bruce Rae was looking after the method development and

specialist tests. As his health failed more of this work came into the area of my responsibility.

It was here in Christchurch in about 1980; that the standardised Thromboplastin reagent we had been working on in Auckland was used routinely for the first time in New Zealand. The use of what became known as New Zealand Standardised Thromboplastin gradually spread until almost all laboratories in the country were using it. At that time it was produced in three centres, Auckland, Wellington and Christchurch, with the three centres working together on quality control and assisting each other in maintaining a supply of reagent if required. Over time and with information gleaned from experience and from a time spent working with the Thromboplastin unit of the Prince of Wales Hospital in Sydney, I developed an equivalent product produced from rabbit brain. Because of the time involved in the process, the production and distribution was eventually given over to a commercial company although the quality control was retained in Christchurch. This reagent was widely used throughout New Zealand until the early 90's when it became obvious that the new recombinant products coming out of the States were superior to anything that we could produce and Coagulon was phased out.

In mid 1983 I was extremely privileged to be invited to spend six months as a research fellow in the laboratory of Professor Inga-Marie Nilsson in Malmo, Sweden. This laboratory was internationally renown for its work on Haemophilia as well as the fibrinolytic pathway, and where, in 1975, Dr Johan Stenflo had discovered Protein C.

The story of the discovery of Protein C and the Protein C pathway is an excellent example of where people noticed the pieces of the jigsaw that did not quite fit and investigated further. For those of you who are unfamiliar with the field of haemostasis, disorders of some part of the Protein C pathway make up the largest group of inherited disorders leading to thrombosis identified to date and include deficiencies of Protein C and it's co-factor Protein S, as well as Activated Protein C Resistance, which is mainly due to an abnormal factor V molecule known as Factor V Leiden.

When he discovered Protein C, Johan Stenflo was on what was essentially a fishing exercise looking at the vitamin K dependant coagulation factors. Using ion exchange chromatography of a precipitate from bovine plasma he separated out four peaks. Peaks A, B and D were shown to consist of the known vitamin K dependent proteins. The protein in peak C, while having a similar amino acid composition to the other proteins was new, and at the time of the initial publication the function of this new protein, Protein C, was unknown. This is in contrast to the discovery of most of the factors of the coagulation cascade, which were discovered in the course of the investigation of a patient lacking the protein, who had presented with a specific problem. This time there was a protein looking for a function.

By the time of my arrival in Malmo in 1984, Protein C deficiency was recognised as a cause of an inherited thrombotic tendency and the laboratory was performing a radioimmunoassay for Protein C using an in-house antibody. I was given the task of developing a functional assay for Protein C. At that time functional assays were beginning to be developed and there were three functional methods that had been recently published by other laboratories. Two of the published methods were similar, using the physiological activator, but both used a synthetic chromagenic substrate to measure the end point. The third method used a physiological end point, the inhibition of clot formation, but used a non-physiological activator. This meant that it was a somewhat clumsy method involving many steps.

We originally set up one of each type of method, and although my supervisor was happy with using the chromagenic assay, I have always had an innate suspicion of chromagenic substrates, preferring

where possible to use the natural substrate, so I was keen to attempt to combine the two systems into one simpler and more physiological method. This way I felt that we could be sure that we were detecting all possible functional abnormalities of the Protein C molecule.

It was when using the method that we finally devised in the routine investigation of patients presenting with a history of thrombosis, that the person performing the tests noticed that there were anomalous results in some patients. Further investigations of these patients by Professor Bjorn Dahlback and his group in Malmo led to the discovery of Activated Protein C Resistance and, as they were beaten in the race to identify the genetic abnormality by Dr Fritz Rosendaal's group in Leiden, the eventual identification of Factor V Leiden.

Had we settled for using one of the published chromagenic assays, APC resistance would not have been discovered in Malmo and as the majority of laboratories performing Protein C assays over the following years were using the simpler chromagenic assay system, one has to wonder when APC Resistance would have been discovered. APC Resistance makes up by far the largest group of patients with an inherited defect leading to thrombosis, somewhere between 20 and 40% of patients with a thrombotic history, whereas all of the other known abnormalities combined make up somewhere in the order of 10%.

The experience of my time in Malmo is one that I look back on with great memories. The development of the Protein C method used many of the skills that I had learned over the years and the eventual outcome was much greater than anything we could have imagined at the time.

Over the following years I was able to travel to a number of conferences in Australasia and further abroad, as well as spending short periods of time in several overseas laboratories. I was given much encouragement and assistance in this by a number of people at Canterbury Health Laboratories. We should not underestimate these types of experiences in expanding our boundaries; they had a major influence on my career.

The early 1990s were a difficult time with changes in management style, cost cutting and industrial unrest. Resources were stretched and trying to find funding for equipment and staffing that were essential to providing a level of service that I could live with, was not forthcoming.

A comment made to me at this time by someone who should have known better, was the beginning of the end of my specialist laboratory career. They said that my problem was, I was trying to provide a five star service when in fact all the medical staff wanted was a three star one. I left medical laboratory science at the end of 1995, and I think that it would be fair to say that at the time I was disillusioned and burnt out.

I still don't know what the medical staff did expect, but the media feeding frenzies over the following years show that the public of this country expected and believed that they were getting the best laboratory service we could provide. Hopefully we have now moved on from that style of management and cost cutting that I do not believe did our profession any favours.

I began the second phase of my career after several years enjoying my life style change in sunny Central Hawkes Bay, when I was asked to take over the Waipukurau Hospital Laboratory following the sudden departure of most of the staff. I thought at the time that it sounded like a bit of a challenge. The last seven years, initially full time in Waipukurau and latterly as a part time locum in Wairoa have proved to be the most challenging, at times the most frustrating, but over all, the most fun of my career to date, with a real feel of coming home to do what I trained for over thirty years ago. I would like to acknowledge my colleagues in Hastings for their patience and understanding as I endeavoured to remember the science of biochemistry and the art of

microbiology that had been dormant for those 30 years!

A number of years ago while on holiday in Milford Sound I got talking to an American Air force pilot and while we enjoyed a launch trip on the Sound we watched a small plane flying around, perhaps taking tourists on a sight seeing flight. This man, who had been telling me that he flew enormous hospital planes and had taken part in the evacuation of US personnel from Vietnam, commented that what we were now seeing was real flying, seat of the pants stuff. That to me is what it is like coming back to the basics of medical laboratory science in a small laboratory. It is seat of the pants stuff and really hands on laboratory work. You don't have to be in a large laboratory to see interesting results and find unusual abnormalities. Obviously you won't find them as often, but in a small laboratory you have the added advantages that possibly you know the patient, probably you collected the sample, you get to see all of the results when authorising them, and in all likelihood you will deliver the report to the doctor.

Much of my laboratory experience predates IANZ inspections, documented methods complete with headers and footers, and to some extent, external quality control programmes. Have these things made a difference? Undoubtedly they have, but my question is, with all of that in place, do we do the basics well? I am sure that we all want to, and mostly we do, but I am not sure that we couldn't do better at times. We are not all going to be in a position where we can discover new diseases or work at the cutting edge of medical laboratory science, but we should do our absolute best to ensure that we don't miss what is in front of us. While large laboratories will naturally find more abnormalities because of the numbers of samples processed, in my experience many interesting findings have come out of small laboratories where the staff noticed and followed up on unusual results.

I don't know how many times over the years I have heard people say "we don't see that here." Sometimes there seems to be an implied, "(I don't think so because) we don't see that here," and other times it is said wonderingly as the speaker appears to be silently asking themselves "Why not?"

I have a few examples, where abnormalities were missed because the laboratories concerned were not following up on unexpected results that they had actually seen. It would be easy to say that the requesting doctors should share some of the responsibility, but that aside, I believe that it is part of our job to ensure that we, as the professionals in the field of medical laboratory science, play our part in following up anomalous results and alerting medical staff to possible causes. In fairness to my colleagues in Wairoa, I would like to make it clear that the following examples are not from that laboratory.

Two patients in their late 70s presented to a laboratory within a few months of each other. The first, a 79-year-old man, was being investigated for possible gout and as part of that investigation the GP had ticked the full blood count box. It was discovered that he had a mildly elevated white cell count of around $17 \times 10^9/L$ with about 70% of the cells being mature lymphocytes. Subsequent cell surface marker results indicated that he had Chronic Lymphatic Leukaemia. A few weeks later a woman, also in her late 70s, presented with a much more elevated white count, at about $50 \times 10^9/L$. The majority of the cells were lymphocytes and some of them were immature. The diagnosis in her case was also Chronic Lymphatic Leukaemia, but in the terminal phase, and she died a few weeks later.

When the past history of both of these patients was investigated it was noted that both of them had had blood taken two or three years prior to this presentation, and at that time both had an elevated white cell count of between 17 and $20 \times 10^9/L$ with an obvious lymphocytosis. In both cases the laboratory that performed the initial tests must have noted this as they had also performed a Paul Bunnell test for glandular

fever. In both cases this was negative, and in both cases the results were reported without comment or suggested follow-up.

This laboratory was enrolled in an external quality control programme that included the examination of blood films. In reviewing quality control blood films, which are expected to be abnormal, and perhaps with a bit of a twist as a trap for the unwary, in general they were able to provide a good differential diagnosis. Why then was the same criteria not part of the examination of patient's blood films? For the same abnormality to be missed twice suggests that the staff of this laboratory were not expecting to find anything. I would like to suggest that if your laboratory's response to a lymphocytosis in an elderly patient is to perform a test for glandular fever, and not consider the possibility of CLL, then perhaps you are missing something.

In another example, an elderly woman had been having a regular blood test and urinalysis performed for several years. On an internal laboratory review it was noted that she always had 2+ to 3+ glucose on the urine dipstick, and that a blood glucose level was not included in her routine blood tests. Her GP was contacted and was most concerned at the possibility that the patient was diabetic. Further investigations subsequently proved this to be the case.

The comment from the laboratory staff when asked about this patient was that she was "always like that." If you know of a patient who has results that are "always like that", in what ever field of laboratory medicine you practice, and you have not asked why, or attempted to find the reason, have you ever asked yourself if perhaps you are missing something?

If the laboratories in your area are seeing a certain disease or abnormality, performing a test or using a culture media that your

laboratory is not seeing, or using, can I suggest that, unless your population base is markedly different, you ask yourself if you are missing something. For instance, if you work in an area with a reasonable sized Maori population you would expect to see a number of patients with a blood picture suggestive of mild iron deficiency anaemia. In the light of recent studies, if you do not expect that a reasonable proportion of them actually have Alpha Thalassaemia, then I suggest that you probably are missing something.

Enjoy yourself over the next few days, make the most of this opportunity to talk to people during the breaks, ask questions, establish links that will help you to do the basics well. Take note of the people here who could help you with answers so that you can be sure that you are not missing anything in your laboratory.

I have attended a number of scientific meetings over the years, often sharing with Jan Nelson. Jan has sworn me to secrecy so I can't share a number of funny stories! However, I will always remember an international conference we attended a number of years ago. One particular presentation sticks in my mind for all of the wrong reasons. I have no idea what that the paper was about, and most of the audience probably could not have told you minutes after its completion either. The Japanese presenter mistook the handheld arrow pointer for a microphone and grasped it firmly in front of his face. We all watched with fascination and not a lot of other attention, as he presented his entire paper with a little arrow of light on the end of his nose.

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Med-Bio Journal Award



Med-Bio offers an award for the best article in each issue of the *New Zealand Journal of Medical Laboratory Science*. All financial members of the NZIMLS are eligible. The article can be an Original, Review or Technical Article, a Case Study or a Scientific Letter. Excluded are Editorials, Reports, or Fellowship Treatises. No application is necessary. The Editor and Deputy Editor will decide which article in each issue is deemed worthy of the award. If in their opinion no article is worthy, then no award will be made. Their decision is final and no correspondence will be entered into.

Winner of the August 2005 issue was Paul Austin from the LabPlus, Auckland for his article "Comparative sensitivities and specificities of two rapid HhsAg detection methods and their relationship to a third generation commercial enzyme immunoassay". *N Z J Med Lab Sci* 1995; 59 (2): 38-40.

Genesis and evolution

Maxine R Reed, FNZIMLS; Biochemistry Specialist, Southern Community Laboratories, Hawkes Bay Ltd., Hastings

Abstract

Challenges and considerations involved in the creation of a Biochemistry department in a new community laboratory are presented. The industry of Medical Laboratory Science is changing rapidly. The competitive nature of the marketplace today requires increasingly efficient utilisation of staff and instrumentation, and a commitment to customer focus. The advantages of fostering mutually beneficial relationships between laboratories and manufacturers, and laboratories and their customers are becoming more evident. Breaking down departmental barriers is coming increasingly important, as is the need to recognise all our customers, not just the traditional ones. These issues are affecting laboratories of all sizes, and the way, in which they choose to, approach them, will dictate their survival.

Successful evolution of a laboratory requires a committed team working in a supportive organisational culture. By using carefully designed processes and teamwork it is possible to achieve these objectives, while creating the value your customers require. By recognising the interrelationship of all areas of the laboratory, it is possible to create quality customer service and enhance the working environment, while achieving continuous quality improvements.

Key words

Community laboratory, customer focus, organisational culture, virtual teams.

Introduction

In the mid 1990's, an outside competitor community laboratory entered the Hawkes Bay community laboratory market with the innovative idea of shipping community laboratory work to Christchurch for processing. Effective use of electronic result transfer and strategically planned shifts allowed the new laboratory to compete directly with the existing community laboratory.

However, by 1996, the newcomers had gone into receivership, and Southern Community Laboratories purchased the residual business. Subsequently pockets of remaining clients were identified around the country, and the decision was made to maintain the service already supplied to these clients, and so shipping of samples to Christchurch continued. During the establishment of business relationships with the newly acquired customers, it became apparent that for optimal service in the Hawkes Bay area, a new laboratory should be commissioned on site. So, in response to customer requirements, Southern Community Laboratory - Hawkes Bay Ltd., opened the resulting laboratory on March 22nd, 1998, with International Accreditation New Zealand (IANZ) accreditation obtained for both laboratory and patient services centres in March 1999.

The objectives of the new laboratory were:

- To retain initial customers, by carefully managing customer relationships with existing clients.
- To expand the client base in the area, by actively competing in the local marketplace.
- To establish and maintain the commercial viability of this venture in the competitive market.

The resulting laboratory was created as a customer driven venture, utilising process centring and virtual teams. Biochemistry specific issues will be examined in more detail.

Environment

To understand the context in which the genesis and ongoing evolution of this laboratory occurred, it is necessary to highlight environmental factors influencing this venture.

Global factors

Medical Laboratory Science, as an industry, is changing rapidly, and from a global perspective, the drivers of change come from a variety of sources:

- Global evolution of health services towards population-based wellness systems (1)
- Downsizing/restructuring/re-engineering of the laboratory coupled with an increasing workload (2-4)
- Integration of "laboratory work" into the provision of "healthcare" by way of closer ties with clinicians and other service providers (2,5)
- Mergers, alliances and acquisitions in supply companies (3,6), and laboratories themselves (7)
- Expanding markets for Point-of-care testing (POCT) and non-invasive technologies (3)
- The introduction of new, more clinically significant analytes, at the expense of now obsolete, less clinically significant tests (3,8)
- Cost containment pressures on the clinical laboratory (3,5-7,9)
- Development of new assays and information handling technologies with associated developments in instrumentation, aiming towards decreased costs, decreased turn-around-times (TAT's), increased capacity and enhanced workstation consolidation (5,6,10,11)

These are examples of ways in which the international marketplace is exerting its effects on local medical laboratory science.

National factors

In 1984, New Zealand's individually managed hospital boards were restructured into 14 area health boards. In 1990, these area health boards were restructured further, resulting in 4 regional health authorities (RHAs) and 23 Crown health enterprises (CHEs). In 1997, the 4 RHAs were merged into one national funding body, the Health Funding Authority (HFA) (7,12,13), and now the most recent round of restructuring has provided the health service with District Health Boards (DHBs). These continued government driven health reforms with their associated funding constraints are compounding the international trend towards downsizing/restructuring/re-engineering of the laboratory. These ongoing health reforms make it increasingly difficult for laboratories to identify their customers, or tailor their services to their requirements.

It must also be noted that International Accreditation New Zealand (IANZ) standards changes are currently being implemented within the industry, alongside the national health reforms.

Local factors

As previously discussed, the local marketplace had previously undergone considerable change. Initially, there were laboratories in the Hastings, Napier and Waipukurau Hospitals, as well as a local community laboratory. The Napier and Hastings hospital laboratories

merged on the Hastings hospital site, and a contract for laboratory services was touted between the community laboratory and the hospital, but this did not come to fruition.

And, as previously mentioned, the mid 1990's brought unexpected competition to the area from a Christchurch based community laboratory. But as with many innovative companies entering into existing competitive markets, their organisational life span was short (14), and by 1996, the newcomer had gone into receivership.

Physical factors

The "Genesis" of a laboratory is not a common occurrence. More often the norm is re-engineering, relocating and/or renovating of an existing operation, infrequently into a purpose built facility. However, in this venture this was not the case and the site chosen was previously a "fast food" outlet.

The most significant "environmental impact" of the site chosen, was NO INTERNAL WALLS in the majority of the laboratory area. Lack of walls challenges traditional thinking in regards to the placement of equipment, and the locating of essentials such as bookshelves and fire extinguishers. This "laboratory void" was complete in that there were also no traditional departmental barriers, either physical or mental.

Focusing on the customer

Stakeholder theory expands the view that a business is responsible only to its owners. Instead suppliers, customers, employees and the community as a whole, are considered to be included in the responsibilities of a business. Recognition of the requirements of these stakeholders and their potential contributions to a business are extremely important, however, they are beyond the scope of this dissertation, which will focus more specifically on customers, both internal and external.

Customers can be defined as those who use the products and services produced by someone else. Customers are the reason for being in business, they define a business, as well as the value of its products and services, and lack of willing customers in a competitive marketplace can lead to the demise of a business (15).

The customer's focus has moved from products and services to the value they can gain from them (16-18), and this must be reflected by a parallel move in the laboratory's focus. By being customer driven, and adopting a customer relationship oriented approach (19), laboratories are better able to understand the value their customers are seeking, by forming mutually beneficial partnerships with them (15,16,19). And as a customer relationship oriented approach involves getting to know your customer's customers (15), medical laboratories are at an advantage in that they routinely do this by dealing directly with patients.

Medical laboratory science is a service industry. By definition, service is the doing of work for another or a community; assistance or benefit given, provision or supplying of a public need. These are all functions that the medical laboratory fulfils, and in doing so the laboratory provides a product, and that product is information.

In this country there are a variety of instruments used by laboratories to provide the "results" or information that the laboratory supplies. And, while accuracy and precision are still important considerations for laboratory workers, the quality of the information itself, in its raw form, is relatively uniform between suppliers. Therefore, it is the ease with which laboratories make this information accessible, and the way in which they tailor the presentation of this information to their customer's needs, that adds value and makes the point of difference between different suppliers.

Staff selection and training, which has been predominantly technical ability and qualification based, must now also reflect the importance

of people handling skills and the ability to be "customer driven" (16). In the past, some laboratories have been guilty of providing a service that was inflexible, unfriendly, and driven by the laboratory's need rather than the customer's needs (19). In many cases laboratory customers were predominantly "hostages" (16), and as "hostages" these customers had little, if any, choice in laboratory service provider. However, as healthcare becomes more community-based and customers more sophisticated, the once "hostage" customers are becoming increasingly aware of the choices they now have in how their healthcare is provided and by whom. And, as more hospital and health services (HHS) laboratories are given access to the laboratory-funding schedule, the choices available to customers will increase.

The laboratory's customers, while still including patients and their clinicians, may also include hospital staff, practice nurses and managers, other healthcare providers, community-based care facilities, reference laboratories, and other staff within the laboratory. And, while some laboratories may have even more diverse customer bases, it must be recognised that each different group has different service requirements, different expectations and perceptions of what value is, and represents different market opportunities to be explored and developed. When viewed in this light, the acknowledgement of the need for customer identification and the development of customer focus (13,20), is imperative for the survival of medical laboratories in the rapidly changing healthcare market.

To this end, the decision to locate an on site laboratory in the Hawkes Bay area, in response to customer needs, clearly stated the intention of SCL-Hawkes Bay, in that this was to be a customer driven organization.

Process centering and organisational culture (21,22)

Having identified the importance of a business's customers, it was necessary to incorporate this customer focus into the way the new laboratory was created. A process centered approach emerged, as the most appropriate approach for the situation (14).

Processes can be defined as groups of related tasks that, when combined, create value for the customer (23), and so the customer's expectations and requirements must be sought, considered and utilised when designing successful processes. Ongoing feedback must also be sought from internal and external customers, to ensure processes continue to meet and exceed customers' expectations and requirements (15,16,19), with documented evidence of this feedback now required for IANZ accreditation.

Designing the complex processes involved in the delivery of each patient's results requires knowledge of all tasks involved from collection of samples, to the final delivery of results in a form acceptable to clinician/patient. On-site staff involved in the creation of this laboratory and its processes gained a thorough understanding of all tasks involved, while utilising their differences as people, with different work "frames of reference" (24). Their personal experiences of previously used processes made them "insiders" with tacit knowledge (25) and experience of specific tasks within a process, while also being "outsiders" with no knowledge of other tasks, but able to offer objective perspectives (23). This diversity enhances the creative potential of process design, while potentially minimizing non-value-adding work (9, 21) and giving ownership of the processes to the staff creating them.

To facilitate ongoing process evolution, mistakes are documented as "Performance Improvement Opportunities (PIO's), analysed, and treated as opportunities to learn and to refine processes. However, tolerance of these mistakes does not mean acceptance of poor performance.

In this laboratory traditional boundaries were redundant, staff needed to work smarter, not harder, and because evolving processes

were big and complex covering a wide range of tasks, all those involved needed to understand the "big picture" (15,20). Because some processes extended beyond the SCL-Hawkes Bay Ltd site, it was necessary to gain knowledge about those parts of the processes performed on different sites within SCL. And thus, on-site staff spent time in SCL, Christchurch, defining their customers' needs, as well as completing the "big picture" of the processes involved. The experience demonstrated how outputs can be customised to customer needs, and gave more autonomy and control of the way processes functioned to the people creating and using them.

The importance of people handling skills, when recruiting staff, is also demonstrated by a process centered operation. Staff with a thorough knowledge of what internal processes can supply are equipped to make decisions relating to service provision at the point of delivery, once they have established what a customer wants and/or needs. This flexibility is supportive of the range of services required by individual customers, and promotes commercial success in a competitive market (17).

Understanding the "big picture" of the processes performed in the laboratory was initially easy for new staff members, because they were directly involved in all processes, but as the venture has grown, new staff are being utilised in more specific areas, and so an overview of organisational processes must be incorporated into the induction of new staff members. Existing staff are also moving into more specialised areas as workload increases, and thus must be kept updated with process changes as they occur.

Staff involvement in process creation also fostered shared understanding amongst staff members in relation to the new organisation, particularly regarding how things were done and the way in which members were expected to behave, essentially ensuring all staff were pointed in the right direction (22). This creation process allowed the formation of local cultural values, whose development is associated with the development of trust, which is key to the success of any team venture. Cultural values also help create an organisational culture, which in turn creates the working environment of a business. An optimal working environment is essential, because even if you have the right people using the best processes, without the right environment they will not succeed (21).

An organization's culture is also sourced from its founders, who foster this culture by the recruitment and subsequent indoctrination and socialisation of like-minded employees, and, by their ability to become role models (22), or "inspirational players" (26). In this capacity they establish and maintain the direction of a company, sharing the dream, creating the future and creating community. Local founders have encouraged the formation of an organisational subculture, specific to SCL Hawkes Bay Ltd. This subculture shares the core values of the dominant culture of its parent company, but for operational and geographical reasons, maintains a strong organisational culture of its own (22).

There are, inevitably, cultural conflicts between sites because different sites operate in different marketplaces with location specific requirements and consequently generate their own organisational subcultures. Potential conflicts can also be exacerbated by a lack of face-to-face contact. However, if "inspirational players" successfully foster a feeling of community, with its associated trust, these potential conflicts can be minimised.

While new staff members must be given an overview of laboratory processes, they must also have cultural values instilled in them, as part of their "socialisation" into the company (22). Failure to do so can affect the way a team functions because it can adversely affect the work environment, and may not only disadvantage new staff, but may also disadvantage the team as a whole, by potentially creating ongoing

problems that could easily be avoided.

Within this culture the ability of staff to update their "mental models" (22), that is the ability to "un-learn" old practices, as well as learn new practices, is invaluable. New staff being socialised into an organization require this ability to "un-learn" previously established ways of doing things. Existing staff members require this ability to utilise opportunities the introduction of new staff offers. That is, resisting "groupthink" (22,24) by re-evaluating current organisational processes, and integrating and mobilising newly acquired personal capabilities within the organisation, where appropriate.

It is of interest to note that the key features of a process-centered operation are compatible with those "organisational learning (22,27)". Open systems thinking, awareness of how what you do and what others do are inter-related, improving individual capabilities, team learning, updating mental models, and a clear strategic direction, are all areas of commonality between these two approaches. "Organisational learning" is important, because if organizations cannot "learn", they will cease to exist due to their inability to adapt to their changing environment (22,27). This approach allows organizations to accumulate competence and capacity, even with staff turnovers, and to maintain flexibility and competency during on-going changes in the marketplace. Like process centering, organisational learning is not an ultimate goal to be reached, but a continuing journey of creating greater value, involving the commitment to creating a culture where learning is rewarded, and experimenting with new ideas to continuously find better ways of doing things is encouraged (16,17,20).

Proximate and virtual teams

A team approach attempts to more fully utilize an organisation's human resources, giving better organisational adaptability and flexibility, and if successful, can increase productivity and improve quality - two important elements of competitiveness (28). However, a group of individuals does not become a team simply because it is declared to be one (21,22). By definition, a work group is a group of individuals that interacts to share information and make decisions to help each member perform within their own area of responsibility (22), thus making work groups more suited in situations where a cross-functional approach is not employed. A work team, however, is defined as a group whose individual efforts produce performance that is greater than the sum of the individual outputs, generating positive synergy through coordinated effort (22), thus making work teams desirable in a process-centred operation.

Teams require training, learning, and trust to function successfully (22). Those members of staff involved in the genesis of the laboratory were to become the on-site, or proximate team of SCL, Hawkes Bay. They had all taken a risk to be involved in the creative process, and had in common the professional attitudes of self-motivation and discipline, sincerity, and enthusiasm, as well as a commitment to do what was necessary to get the job done (22). By dealing with the technical issues associated with creating the processes first, rather than concentrating on the social components of team-building, the proximate team was successfully building up trust levels while seeing results (29), creating a shared sense of achievement coupled with a growing respect for other team members (21). And, while forming the proximate team of SCL, Hawkes Bay, members were also becoming part of the "virtual team" (22,31) of SCL as a whole.

Virtual teams are groups of people who work closely together, even though they are geographically separated, by utilizing communications technologies, in this case, E-mail, a shared database, the Telecom Centrex system, cell phones, teleconferences, faxes, and internal mail. In a competitive market, virtual teams allow organizations to bring their employees closer to the customer to respond to customer needs, and

in doing so, bring diverse skills and expertise to customise solutions to meet market demands (31). Like proximate teams, the development of trust between virtual team members is imperative, but is more difficult, due to the lack of paraverbal and non-verbal cues (22,29), and the limited social context of the team. Ideally, "rich" media, those in which paraverbal and nonverbal as well as verbal cues are transmitted (29), are needed in the "forming" stages of team creation. To this end, all original staff spent time at SCL, Christchurch, receiving training, and meeting other team members. The time and money invested in bringing team members face-to-face allowed trust to form before communications became predominantly vocal and electronic. This enabled staff to develop working as well as social relationships with other staff within the team, reducing feelings of isolation from the rest of the company (22,31), and as already discussed, aided process design by facilitating the identification of requirements of internal customers by better understanding what internal customers need, and why. By developing relationships with internal customers, it is possible to remove non-value adding work, and some cases remove tension from the work place, purely by customisation of outputs to be more compatible with their needs.

As with all teams, virtual teams require ongoing team building, and the addition of new members, either on-site, or in other areas of the company, can create problems. Trust is a property of a relationship between parties (22,29,32), so when a new member is introduced into a proximate team, trust develops as the relationships between the new member and the existing members develop. If the new member is also to be part of a virtual team, but for financial/operational, and often purely practical reasons, cannot spend time face-to-face with other virtual team members, the relationships, and therefore the trust required, take longer and are often harder to develop.

When examining the operation of virtual teams, it is also necessary to consider the location of face-to-face interactions. Initial proximate team members continue to utilize conferences, users groups, special interest groups (SIGs), and visits by staff to continue the face-to-face component of on-going team building. The initial time spent in Christchurch gave an understanding of the processes used and the work environment of their virtual "team mates", which is utilized in interactions with them. In most cases, however, there is not a reciprocal understanding of processes and work environment. This is also the case with other SCL laboratories around the country, and vice versa. While it is not practically or financially viable to have all staff members visit all sites within the company, lack of understanding of how and why different sites operate the way they do, can hinder the effectiveness of virtual teams.

It is of interest to note, that IANZ requires bi-annual pathologist visits as part of its registration requirements, even though this laboratory has consultant pathologists members in functioning virtual teams overseeing remote site operation, and having far greater clinical input on a day-to-day basis than could be obtained in two visits per year. This raises the question of whether the quality of consultant pathologist input should be under scrutiny, instead of the quantity of on-site time.

Centres of excellence

In addition to the virtual team, which predominantly involves the company's Christchurch operation, and the on-site proximate team, there also exists within the company as a whole, what could best be described as discipline specific "Centres of Excellence". Each discipline has its own consultant pathologist or pathologists, who are accessible to staff and clients alike, who act as the "inspirational players" for the discipline and who network with other staff within the discipline, to form the "Centre of Excellence". Information is shared in the form of

circulated journal indexes, e-mail, articles of interest, circulating slide sets, and memos, as relevant issues arise. Method and instrument comparisons and evaluations are more easily achieved with multi-site interaction. Similarly, if a specific problem occurs, members can contact individuals with the required knowledge and experience for the relevant information and advice. In the discipline of Biochemistry, teleconferences, intranet pages, and correlation of external survey results are also utilized by the discipline as a whole. Therefore, while not strictly including all these additional staff members into the "virtual" SCL Hawkes Bay Ltd. team, it is still possible to access and utilize their collective knowledge and experience. The degrees to which these "Centres of Excellence" are utilised are quite discipline specific, however, in Biochemistry, members are routinely interacting on a day-to-day basis.

Biochemistry

The task of setting-up Biochemistry was essentially three-fold:

1. To design new processes from those previously experienced, removing as much non-value-adding work as possible.
2. To prevent non-value-adding work entering the new processes due to loss of focus.
3. To ensure that the "Biochemistry" processes, as such, were efficiently integrated into the total delivery of a complete service to our clients.

The core laboratory (33), workstation consolidation (34), instrumentation, test selection, and quality management, were all examined closely as a means to the accomplishment of this task.

The core laboratory and workstation consolidation

For the success of this operation, it was necessary to construct the laboratory as a core laboratory, with close linking between Biochemistry and Haematology, thus reducing the potential for staff and equipment redundancies. The original floor plan, however, did not fully reflect this concept. The initial layout was generated off-site by senior staff at other locations within the company, utilising movable benching to maintain flexibility, and was a starting point to work from. At the time of setting up, however, the moving of equipment and large benches was not a high priority and so the initial layout remained unchanged. Since then it has evolved and the rearrangement of benches and equipment over time has allowed the development a flexible, workable laboratory area, through which specimens and information "flow", and in which staff are more efficiently utilised.

Again, the lack of walls has been advantageous, allowing contact between different areas, while maintaining separate workspaces. The Haematology and Biochemistry areas have been adjoined to facilitate staff sharing between the areas aiding continued evolution towards a more user-friendly design and supporting ongoing cross training. Utilisation of ceiling mounted power, phone and computer connections, combined with bench integrated multiboards, have also enhanced the potential to rearrange the entire operation, with the exception of one analyser, which has its waste physically plumbed in. The advantage of minimising biohazardous waste handling through plumbed in waste, must be considered versus the loss of flexibility due to permanency of instrumentation placement.

The core laboratory concept also highlighted the need for a centralised specimen reception or central processing area (35). This area has given us the greatest opportunity to utilise staff's tacit knowledge in the refinement and evolution of its processes. The quality of all results produced in the laboratory, regardless of department, are completely dependant on the successful functioning of this area. Its processes are labour intensive, susceptible to errors, and the work itself is repetitive (36). In a much larger operation, this area would

lend itself to automation, but in this situation the chosen plan of attack is to minimise the sample separation as much as possible. This is achieved by careful education of patient services centre staff in sample requirements (35), that is collecting separate tubes for separate locations where possible, and by consolidating Biochemistry workstations and running processes in such a way as to allow a single primary tube to be used sequentially on all main analysers, if multiple tubes are not received. This minimises the need for in-house aliquots, while reducing the need for forwarding aliquots to other sites, unless absolutely unavoidable.

The use of a single primary tube, run serially on the required instrumentation can potentially slow down TAT's, but reduces specimen handling errors, whereas the use of multiple primary tubes, or alternatively primary tubes and aliquots allows tests to be run in parallel on the required instrumentation, thus improving TAT's (11,34). While the use of multiple primary tubes, would be ideal, it is currently felt that the loss of TAT is compensated for by the reduction in potential specimen handling errors by minimising aliquot use where possible.

Again, the successful running of specimen reception processes requires an understanding of the "big picture" by those staff utilised in the area, which is enhanced by our high level of cross-training. There are difficulties in ensuring the continued updating of all staff working in this area, as changes to processes in different areas of the on-site laboratory, as well as at off-site locations, have a direct bearing on how effectively the current processes perform. Utilisation of a continuously updated specimen collection guide currently provides the most successful transfer of collective knowledge between the different shifts operating.

Workstation consolidation is an obvious choice when trying to remove "non-value-adding" work from processes. By consolidating assays onto the least number of analysers, it is possible to organise the workplace according to process flow rather than departments (10,34), combining and simplifying actual processes, minimising space requirements, and eliminating unnecessary activities (9,11,34) such as additional transport and aliquotting of samples.

Initially the Biochemistry analyser configuration consisted of a "carousel" type analyser and a "racking" analyser. The advantages of moving to both racking analysers, or ultimately a modular system (10,36), that essentially combines both current analysers, are evident. As previously mentioned, the operation is currently not of a size that would warrant the utilisation of a "Specimen Processing System" (10) for pre-analytical sample handling, but by adopting a racking or modular approach to the analytical phase, it would be possible to further increase test range and workload capacity without major increases to staffing levels, and further eliminate the need to split samples for different workstations (36). And while this operation is also too small for a "Storage and Retrieval System" (10), it does utilise its computer systems accessioning program to make retrieval of specimens for additional testing easier and more efficient.

The recent upgrade to two racking analysers has allowed the minimisation of specimen manipulations required for each sample, thus enhancing operator safety (34), and has enabled the refining of current processes, allowing analyser racks to be loaded at Specimen Reception, rather than having specimens loaded into racks and then transferred onto the analysers, once in the Biochemistry area. This would also be possible with a modular system.

The move to a modular system would also require only one procedure manual (10,11), rather than the two currently in use.

Instrumentation and test selection

Labour is the largest single cost for a laboratory (37). By selecting the right automation options, matched to the laboratory, its market

environment and its processes (10,11,38), it is possible to maintain minimum, but adequate staff levels, while improving service, and controlling labour costs. The selection of the correct instrumentation involves many facets, and many of the considerations facing larger operations are just as applicable to small to medium sized laboratories. The following questions are among those for consideration in the selection process:

- What is the likely test range that will be required?
- Where and how will the instrumentation fit into the processes being designed, or redesigned in existing laboratories (5,39).
- What instrumentation alternatives are available for the potential workload and test range required, including new or reconditioned alternatives (39)?
- How user-friendly and reliable is the instrumentation, and how good is the backup from the supplier (39)?
- Is the analytical performance of the instrumentation of an acceptable level (38)?
- Is it possible to interface the equipment?
- Are routine and stat TAT's compatible with the desired level of service required?
- What is the expected life cycle of the instrumentation, and, will it be possible to trade up, trade in or resell used instrumentation (38)?
- What is the potential life expectancy of technologies used on the instrument, and what is the likelihood of an expanding test range?
- What capacity is available for increased workload and increased test range (37)?
- On comparative cost analysis, which of the available alternatives is acceptable (39)?
- What financing options are available; eg operating lease or rental, reagent rental, cost-per-reportable test?

Yet again, it becomes apparent that even the issue of instrumentation selection is not able to be viewed in isolation because of its interrelation with many other facets of the laboratory from proposed test range to impact on workflow. Previous experience of both instrumentation and supplier service (instrument support and consumables supply) can be accessed and in this situation, bulk deals for commonality of instrumentation within the company, were also able to be utilised.

Here, the selection of Biochemistry analysers revolved around reliability (of both instrumentation and supplier), ease of use, potential for expanding workload and test range, limited "hands on" operator time required, and nationwide commonality within the company of main analyser supplier, which, when common reagents are used, gives consistency of reference ranges across sites.

In any situation of relative isolation, the importance of instrument reliability and good supplier backup become instantly apparent. Good communication with suppliers allows them to gain an understanding of how your business runs, and how their service to you as customers affects your customers. While global changes in healthcare are putting the squeeze on laboratories financially, in vitro diagnostic manufacturers are also feeling the effects (3,5), thus by sharing feedback, both positive and negative, with suppliers, it is possible to develop mutually beneficial relationships (20), giving suppliers the opportunity to tailor their services to their clients requirements. By not communicating their needs and expectations, laboratories can potentially put themselves and their suppliers at a commercial disadvantage.

Ease of use and limited required "hands-on" operator time is desirable in any cross-trained environment. This ease of use covers the whole range of manipulations of the analyser, not just actual

result generation. Properly trained staff must be able to perform maintenance and calibration procedures without unnecessary difficulty. And, as with "walk-away" ability, this is especially important in on-call and shift situations.

The importance of interfacing between analysers and the laboratory information system (LIS) was realised at a very early stage in the genesis of this laboratory. Interfacing improves productivity, by enhancing the ability to do more with less, and improves quality by minimising transcription errors (40) and sample mix-ups, by utilising bar-coded specimens. It allows the refining of processes to remove the non-value-adding work of manual entry, and relieves stress on staff members, and for these reasons, should be considered as a potential source of quality improvement in even small laboratory operations. After initially working without interfaces, American Society for Testing and Materials (ASTM) interface standards (41,42) based bi-directional interfaces (39,41) for the Biochemistry analysers, and a unidirectional interface (40) in Haematology, were introduced after several months of operation.

As with any instrumentation, reliability and support of interfaces (as well as the actual LIS) is crucial to the successful operation of any laboratory, but especially for those in isolated situations. The Biochemistry department began with a relatively conservative test range, covering those tests essential to everyday community work, but not extending to slightly more esoteric tests, mainly due to the low numbers of these test request originally received. By selecting instrumentation with more capacity than was needed initially, it was possible to expand the test range and absorb the increasing workload as required, without major impact on the processes already in place. In fact, by adding to the on-site test range, not only have improved TAT's service been achieved, but original processes have been refined and have evolved, by removing the non-value-adding work of additional aliquotting and dispatching of samples to other laboratories.

Commonality of instrumentation gives additional back up in cases of instrument failure, without (in most cases) alteration of reference ranges. Reagent shortages are also predominantly covered in this way if the manufacturer cannot meet local needs, and as already discussed, trouble-shooting becomes less stressful, with support within the company, as well as from the supplier.

The test range offered by a laboratory should allow clinicians to interact with and treat their patients more effectively (43). It should improve patient outcome and improve healthcare delivery, as well as being cost-effective. In fact, a protocol for medical audits of self-sustaining laboratories proposed by a working party of the Swedish Society of Clinical Chemistry, states, *"the selection of analytes shall be a joint concern for the laboratory and its customers and regularly reviewed to meet the needs of health care"* (44). And, while the traditional considerations of practical and analytical performance parameters still require consideration (45), improved healthcare cost-effectiveness has, and will still, become a more dominant driver of assay use (43).

By improving TAT's it is possible for clinicians to deliver healthcare to patients more rapidly. It may also be possible to reduce the number of patient complaints, and exceed the patients and clinicians expectations, thus supplying excellent customer service (46). Consequently, improving TAT's, and more effective utilisation of equipment, have driven the addition of tests to the existing range, while some assays have been relocated from one instrument to another to improve analytical performance. Conversion problems, such as changes in reference ranges, and the presence of interfering substances in some patient samples, eg macroprolactin and its differing effect on different immunoassay systems have been encountered (47-50), and good communication within the company, and with customers, have

minimised the impact of such change-associated problems.

Having identified the test range to be supplied, those tests that will automatically be viewed as "urgent", in addition to those requested urgently, need to be identified, and processes for the handling of these samples need to be incorporated into the existing processes to ensure their adequate identification and handling. Again, good communication is the key, in this case between staff performing the Specimen Reception processes, and their internal customers, the department specific staff. Often, these are the same people, but clear communication of urgent specimens is still a priority. Also to be considered, when deciding on the range of tests to be supplied, is the potential cost of, and availability of external quality control programmes.

Quality management

Quality, by definition, is "conformance to the requirements of users or customers (51). To successfully manage quality, a complex blend of meeting and exceeding customer expectations, the right organisational culture, a team focus, having "inspirational players", co-operation with suppliers, continuous improvement, and a system of standards and measurement, is required (54). While many of these elements have already been discussed, the measurement system, which can be translated in Biochemistry terms as quality control (QC), requires mention.

Often in Biochemistry departments, the focus of quality is predominantly on QC. The drive to gain better accuracy and precision on measurements within the laboratory, and gain the best results on external proficiency surveys, can become the main goals, at the expense of the determination of what constitutes acceptable analytical performance based on the test result's use (6,53). And, while there is room for improvement in the analytical quality of most assays (54), patients cannot be protected purely through accuracy and precision in the analytical phase of the testing process (55). Quality control is designed to control process variation, but it is only part of the quality management of the laboratory. It cannot detect or prevent most mistakes or errors. Errors in the pre and post analytical phases are likely to compromise the usefulness of laboratory results in patient care more often than errors in the analytical process (54-56). Therefore, it is important to manage the quality of the laboratory's pre and post analytical processes, as well as those of the analytical phase, by understanding and designing these processes in such a way that reduces complexity (57), and prevents or stops errors occurring (58). Again, the use of PIO's to investigate errors and thereby further refine processes, is an essential tool utilised as part of this laboratory's Quality Management.

Growing awareness of interferences in immunoassays from a variety of sources, including heterophile and anti-animal antibodies, drugs and drug metabolites, rheumatoid factor, and cross reacting substances (59,60) is adding a new quality challenge to laboratory analysis. Vigilance by laboratory staff may help identify potentially anomalous results, but the best tool available to overcome these "interference issues" is ongoing communication with clinicians (60,61). Laboratories can provide up-to-date information to clinicians regarding test limitations and performance, while clinicians can provide information regarding the administration of potentially problematic treatments, full clinical details, and query results that do not fit the clinical picture. By actively seeking and fostering this dialogue, the laboratory can improve its ability to manage the quality of the results it produces, thereby improving the service it offers to both clinicians and patients.

QC relies on having optimised and stable testing processes. With new analysers and assay systems being introduced all the time, the industry as a whole is hoping that the analytical quality of these

systems will also be improved, but this cannot be assumed to be the case, as the introduction of these new systems makes it potentially possible to produce more bad results faster than ever before (62). Correlation studies only involved correlation coefficients, slopes and intercepts, in many laboratories in the past, but growing awareness, understanding, and accessibility of the statistical tools available today, is enabling laboratory staff to better assess the claims of these new assay systems (63,64), and make informed decisions regarding the analytical quality, and QC requirements of these new systems.

Conclusions

The genesis and evolution of a new laboratory in an existing market, with established competitors, who have already withstood direct competition from an outside source, is clearly a challenging proposition. The creation of this laboratory has demonstrated the advantages of customer focus and process centering when aiming to retain customers and develop mutually beneficial relationships with them. A supportive organisational culture has encouraged a team environment involving all areas of the laboratory, and the drive to continuous quality improvement and improved customer service has resulted in an expanded client base and sustained commercial viability.

It has also demonstrated the potential advantages and difficulties involved in the use of virtual teams, and the need for closer relationships between suppliers/customers and the laboratory industry as a whole.

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60 Years of Continuous Publication Journal Prize

In 2006 the Journal will celebrate 60 years of continuous publication. To celebrate this memorable occasion, the NZIMLS will award a special prize, worth \$500, for the best case study accepted and published in the Journal during 2006.

Case studies bring together laboratory results with the patient's medical condition. Many such studies are presented by our professional members at conferences and SIG meetings, yet rarely are submitted to the Journal. Start thinking and planning now to submit your interesting case study to the Journal. Not only may you win this special prize, but definitely will earn you CPD points. As all articles in the Journal are peer-reviewed, start thinking about submitting during the latter half of 2005. Please feel free to contact the Editor, Deputy Editor or Members of the Editorial Board if you want advice or guidance.

You must be a financial member of the Institute (Fellows, Members and Associate Members) during 2006 to be eligible. No formal application is necessary. All case studies published during 2006 (April, August and November issues) will be considered. The Editor, Deputy Editor and the President of the NZIMLS will judge the published case studies in December 2006. Their decision will be final and no correspondence will be entered into.

Xenotransplantation and human rights

Warren Lindberg.
Human Rights Commission, Wellington

Fear of the strange and the shock of the new affects all of us from time to time, particularly when complex issues are involved, which get to the very essence of what it is to be human.

Xenotransplantation involves transplanting living cells, tissues or organs between species, and from animals to humans. The word 'xenotransplantation' comes from the Greek word *xenos*, meaning stranger, hence xenophobia.

Two international human rights declarations have been created specifically to deal with the challenges presented by advances in knowledge about human genetics. The principles in those declarations are very relevant to the dialogue about xenotransplantation.

The 1997 *Universal Declaration on the Human Genome and Human Rights* and the 2003 *International Declaration on Human Genetic Data* emphasise that human dignity makes it vital not to reduce individuals to their genetic characteristics and to respect their uniqueness and diversity.

A declaration on bioethics and human rights, emphasising the need to promote respect for human dignity and human rights in decisions or practice involving bioethical issues is also being prepared by UNESCO. One aim of the draft declaration is to recognise the responsibilities of humans toward other forms of life in the biosphere including other humans.

The Human Rights Commission supports the incorporation of a human rights-based approach in the dialogue on xenotransplantation.

Ensuring that the broad range of views on xenotransplantation are heard, whether they have a cultural, spiritual or ethical focus is key to this approach. This derives from article 27 of the Universal Declaration of Human Rights, which states that everyone has the right to share in scientific advancement and its benefits. Specifically, a human rights based approach:

- Links decision-making at every level to the *agreed human rights norms* at the international level as set out in the various human rights covenants and treaties.
- Aims to identify all those whose rights are affected by the issue and where there is conflict, seeks to balance the rights in the way that maximises respect for all rights-holders.
- Places emphasis on *participation* of individuals and groups in decision-making.
- Introduces *accountability* for actions and decisions, which can allow individuals and groups to complain about decisions adversely affecting them.
- Seeks *non-discrimination* among individuals and groups through the equal enjoyment of rights and obligations by all.
- *Empowers* individuals and groups by allowing them to use rights as leverage for action and legitimising their voice in decision-making.

Xenotransplantation gives rise to a number of important human rights issues. These include how New Zealand would deal with any possible public health risks associated with xenotransplantation. How would we balance the right of individuals to benefit from the new technology with protection of the public's health? If New Zealand does not permit xenotransplantation how should it treat those who have received it elsewhere? What does it mean for Maori as individuals and collectively and what is the relevance of the Treaty of Waitangi?

There is also the possibility of discrimination against those who have benefited from a transplant, and may, as a result, carry organisms capable of causing illness, or as is more likely to be the case, organisms suspected or assumed or believed to exist. The Human Rights Act 1993 prohibits this type of discrimination.

It is important that people are informed about the complex issues involved in xenotransplantation in order to encourage a robust public debate.

The Commission is currently working on its contribution to the dialogue being conducted by Toi te Taiao: the Bioethics Council on xenotransplantation. The Commission will post its contribution to the dialogue on its website www.hrc.co.nz in June.

Address for correspondence: Warren Lindberg, Human Rights Commission, PO Box 12-411, Thorndon, Wellington.

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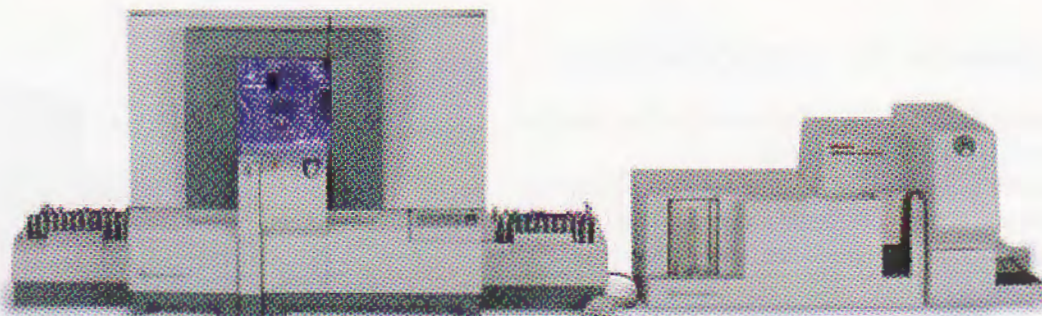


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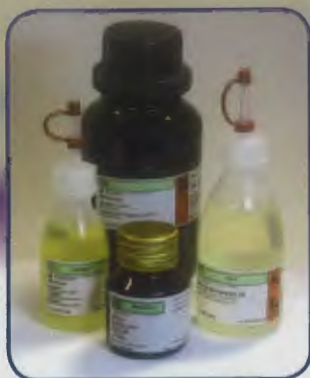
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THE Pacific WAY

Planning is underway to celebrate the 25th year of the Pacific Paramedical Training Centre with a function. This will take place in October with old and new friends of the PPTC meeting at the Wellington School of Medicine.

The first nine months of 2005 have been eventful for the PPTC. Two courses have been held; a Laboratory Management Course in March and a Blood Bank Technology Course in August.



Marie Pio from Vanuatu (left), John Dagger Tutor of the Blood Bank Technology Course and Theresa Tatuava from the Cook Islands

The Regional External Quality Assessment Programme continues to grow and now has 27 laboratories participating in the programme. 21 of these are National Laboratories whose participation is supported by WHO, 19 in Pacific Island countries, three in Laos PDR and one in Thimpu, Bhutan. The Laboratory at the Suva Private Hospital has joined the programme, and along with the laboratories at Lae (PNG), Qui Nhon Provincial Hospital and Bong Son (both Vietnam) are sponsored by private arrangement.

The Centre schedules the dispatch of three programmes each in Microbiology, Haematology, Infectious Diseases Serology and Blood Bank Technology and two in Biochemistry. The PPTC again received a contract from WHO to assist with the financing of the programme and in addition both NZAID and the NZIMLS continued their financial support without which this programme would not be able to operate.

The programme co-ordinators: Clare Murphy, Clinical Biochemistry, Philip Wakem, Haematology, Tirath Lakshman, Blood Bank and John Elliot, Microbiology and Infectious Diseases Serology, put in a significant amount of time into the programme which goes a long way in improving the quality of medical laboratory technology in the Pacific.

Late in 2003 the Centre was approached by WHO in Suva and invited to prepare a pilot course for Laboratory staff to be delivered through their newly introduced distance learning project, The Pacific Open Learning Health Net - www.polhn.com. Computer laboratories have been established in 10 Pacific countries and Health Professionals from these countries have been offered courses for their continuing education. The first course for Medical Laboratory Technicians was on the Laboratory Diagnosis of Diarrhoeal Diseases covering all the common bacterial causes of diarrhoea. The course 'went live' in September 2004 with registrations from students in five Pacific Island countries. The course concluded early in January 2005 with five of the students having completed all seven assignments.

This year a Clinical Biochemistry Course Distance Learning Course has been run. The course was written by Ian Tompson from Wellington Hospital Biochemistry Department. The course was divided into six modules covering subjects such as Carbohydrates, the Heart and Electrolytes and Acid Base. When students finished each module there were questions to be answered which when completed were emailed to the PPTC where their tests were marked and results emailed back. 18 students from 6 Pacific Countries completed the course. Ian is to be congratulated for his skill at creating something uncomplicated and easy to follow which will be of great value to the students who completed the course. If there is enough interest this course may be repeated again in 2006.

The next Course to be run via POLHN will be Laboratory Management and Quality Systems course scheduled to commence in the first half of 2006.

Late in 2003 the Centre was approached by the Palau Ministry of Health to recruit a Medical Laboratory Scientist for a one year contract to assist in improving the services offered by the Laboratory in Palau. The consultancy was also to have a special emphasis on training and the development of a training course which could be expanded to become a regional course for Laboratory Technicians from the US Associated Pacific Island Countries.

The Centre was successful in recruiting Nicole Beamish from Wellington Pathology who commenced work in Palau in March 2004. During the year she successfully conducted training courses in Blood Bank Technology and Parasitology as well as working hard to improve the quality of services offered by the laboratory. Nicole returned to New Zealand early in 2005 and is back working at Wellington Pathology as Acting Charge Scientist in Microbiology.

The Centre has for the past 2 years been acting as a reference laboratory for the rechecking of AFB smears from several Pacific island countries. The PPTC has been officially appointed by the WHO Stop TB Programme to be responsible for the AFB EQA for all countries in the Polynesia group plus Kiribati. This programme involves the rechecking of several hundred AFB smears every year.



Participants, tutors and friends at the Certificate Presentation for Blood Bank Technology Course held 2 September 2005

Christine Storey
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NZIMLS President's Report - 2005

Those who work in the profession of Medical Laboratory Science (MLS) know that change is an integral part of the profession. As the New Zealand Institute of Medical Laboratory Science (NZIMLS) renews itself for the 60th year of its existence in 2006, it is timely to reflect upon changes within the profession since its inception. The Institute's history dates back to 1945 when the New Zealand Association of Bacteriologists was first formed in Wellington. In 1959 the Auckland branch of the Institute formed as Technologists sought a greater need for continuing education. Other historical events have included the formation of the Medical Laboratory Technologists' Board (MLTB) in 1964 and the introduction of Medical Laboratory Technologist registration in 1973. In 1988 the Medical Laboratory Workers Union formed and the industrial affairs portfolio of the Institute split from that of the professional affairs. After many years of trying, two NZ Universities commenced degree training for the profession in 1992 and the apprentice-style training programmes of the past were replaced with fulltime University programmes.

Changes to the profession over the years, reflect changes in the industry, the desire for higher level education and a growing professionalism and maturity. These events have led to the formation of the Special Interest Groups (SIG's), changes to the NZIMLS Fellowship, the website development, and the formation of the Institute's Competency and Professional Development programme.

As it is with any organisation the good and bad times come and go and I recall a time in the recent past when the financial viability of the NZIMLS lay in the balance. This came about with increased costs of running the Institute, a falling membership and a decreased income from the lack of an Annual Scientific Meeting. The good news today is that the Institute has made significant progress forward so that today the accounts reflect a very satisfactory state of financial affairs of the Institute.

The 59th year of the NZIMLS will in my view also go down as a watershed year for the Institute. In this last year, introduction of new regulations governing the health professions in NZ has brought about a big change in the profession. At the official launch of the HPCA Act at Parliament buildings in 2004, the Minister of Health announced that the legislation set new standards for practice as a health practitioner in the developed world. She also went on to say that other nations would be watching the impact of the Bill on both the health professions and the public of NZ. Subsequent changes mandated by the Medical Laboratory Science Board have established scopes of practice for the profession and the registration of Scientific Officers and Laboratory Technicians. New also in the last year is the need for registered MLS to demonstrate laboratory competence prior to the issue of an annual practising certificate (APC). This has required practitioners to belong to a competency or recertification programme which will be used by the MLSB to assess a practitioner's competency to practice. Since the introduction of this requirement the NZIMLS has enrolled approximately 1520 scientists (and some technicians) into its Competency and Professional Development programme. This is from an estimated total of 1700 people who will be required to enrol in this or a similar programme in 2005.

The embryo of today's CPD programme formed in the early 1990's as the Maintenance of Laboratory Standards (MOLS) programme. This programme was devised by the NZIMLS Council of the day and was initially administered by the MLTB. In 1998 the responsibility for running the programme shifted to the Institute and the programme underwent a review. As part of this, the programme was renamed

the NZIMLS Continuing Professional Development (CPD) programme. Another review three years later saw the programme re-released as the Competency and Professional Development (CPD) programme that we have today. The CPD programme has evolved over the years and is the only programme in NZ that mandates a compulsory evaluation of laboratory competencies. It is also the only programme that utilises the internet for paperless submission of points, practitioner record retrieval, and administration of one's CPD record. The programme awards points for a wide range of MLS activities and sets a realistic target of 100 points per year for both part and fulltime Scientists. The CPD programme is available to all Scientists, Scientific Officers and Technicians regardless of discipline or membership of the NZIMLS, and is currently the only programme accredited for use as a re-certification programme with the NZ Medical Laboratory Science Board.

Over the last year it has been very pleasing to see the number of persons who have joined the CPD programme at the same time taking advantage of the benefits of NZIMLS membership. From the Life, Fellow, Member, Associate, non-Practising and Honorary membership categories, which totalled 879 in August 2004, Institute membership now exceeds 1720 and continues to rise.

For the future the Institute is committed to the provision of both continuing education and the CPD programme. It is thanks to the work of the past and present Councils and the ongoing support of the Membership that we find ourselves in a position whereby we are able to provide such a comprehensive package. It is perhaps no surprise the CPD programme has achieved such widespread acceptance among the profession.

Finally it is my pleasure on behalf of Council and the Members of the NZIMLS I extend a welcome to the many new Members of the NZIMLS and invite you to get involved in your professional body. I hope that you find satisfaction in being part of an organisation that exists to promote the profession of MLS in New Zealand.

In closing I would like to state that the last year has been a very strong one for the Institute. Council has been most encouraged by this and we look forward to your continued support in the years ahead.

Chris Kendrick
NZIMLS President



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Minutes of the 61st Annual General Meeting held at Christchurch Convention Centre, Christchurch on Wednesday 17th August 2005 at 7.30am

Chairman

The President (Mr C Kendrick) presided over the attendance of approximately 74 members.

Apologies

Motion:

Moved R Allen, seconded K Taylor

That the apologies from Warren Dellow, Michael Legge and Rob Siebers be received.

Proxies

Motion:

Moved J Wypych, seconded J Whimster

That the list of four proxies as read by the Secretary be received.

Carried

Minutes

Motion:

Moved A Buchanan, seconded R Allen

That the Minutes of the 60th Annual General Meeting held on 29th August 2004 be taken as read and accepted as a true and correct record.

Carried

Motion:

Moved R Hewett, seconded A Buchanan

That the Minutes of the 60th Annual General Meeting held on 29th August 2004 be confirmed as a true and correct record.

Carried

Business arising

Nil.

Remits

Motion:

Moved K Taylor, seconded R Allen

That Policy Decision Number 3 be reaffirmed

Policy Decision No 3 (1973): Policy Decision No 3 (1972): Council will make and administer awards to members of the Institute, the details of each award will be recorded and may be amended from time to time by resolution of Council. The summary of these details shall be published annually in the Journal.

Carried

Motion:

Moved D Robertson, seconded A Buchanan

That Policy Decision Number 5 be reaffirmed.

Policy Decision No 5 (1978): That medical supply companies should not be approached to aid in the finance of Branch or Special Interest Group meetings; companies may be invited to Regional Seminars and although donations may be accepted money is not to be solicited.

Carried

Motion:

Moved K Beechey, seconded Gloria Grossley

That the NZIMLS resigns its membership of the International Federation of Biomedical Laboratory Science.

Carried

Discussion:

- John Elliot, PPTC acknowledge that the IFBLS does have an aid group but that it has been ineffective within the Pacific region.
- C Kendrick advised that the northern hemisphere dominates the IFBLS and that a number of countries including the UK and Australia had pulled out of Membership of the IFBLS. Individuals can however continue Membership of the IFBLS if they wished.

Motion:

Moved J Wypych, seconded J Whimster

That the amended Rules of the NZIMLS be adopted. During 2005 Council reviewed the NZIMLS rules. Since the last review in 1994, there have been a number of changes to the profession brought about in part by the Health Professions Competency Assurance Act of 2003 but also in the way in which the NZIMLS now operated.

Carried

President's report

Motion:

Moved R Hewett, seconded K Taylor

That the President's Report be received.

Carried

Annual report

Motion:

Moved A Buchanan, seconded R Allen

That the Annual Report be received and adopted.

Carried

Financial report

Motion:

Moved R Hewett, seconded J Wypych

That the Financial Report be received and adopted.

Carried

Election of officers

The following members of Council were elected unopposed:

President	C Kendrick
Vice President	R Allen
Secretary/Treasurer	R Hewett
Region 1 Representative	L Glogoski
Region 2 Representative	T Mace
Region 3 Representative	J Wypych
Region 4 Representative	K Taylor
Region 5 Representative	A Buchanan

Answers to HSIg questionnaire

1. Inappropriate oxidation of the ferrous iron found in the haem group of haemoglobin results in a dysfunctional molecule incapable of oxygen exchange under physiological conditions. This leads to anaemias known as anaemias of oxidised haemoglobin.
2. (i) The presence of haemoglobin M; (ii) methaemoglobin reductase deficiency; (iii) toxin-induced production of methaemoglobin.
3. False.
4. Some oxidation of Fe^{2+} to Fe^{3+} does occur. This is due to intermittent discharge of oxygen as a superoxide ion (O_2^-) leaving the haem iron in the Fe^{3+} state. Methaemoglobin reductases in the red cells ensure that the proportion of methaemoglobin does not exceed 1%, above which will result in anaemias of oxidised haemoglobin.
5. Globin chains aid in stabilising the haem-oxygen complex through the formation of the haem pocket. An amino acid substitution will result in the structural alteration of the haem pockets which leads to spontaneous oxidation of the Fe^{2+} ion embedded in haem. Such variants give rise to haemoglobin Ms. Mutation, substitution, His87, Asn.
6. True.
8. It is caused by a deficiency of reduced nicotinamide adenine dinucleotide (NADH)-cytochrome b5 reductase (cytb 5r).
9. Membrane bound form of cytb5r can be found in the endoplasmic reticulum and mitochondrial membrane in red cells. Cytb5r participates in desaturation and elongation of fatty acids, biosynthesis of cholesterol, and P-450-mediated drug metabolism.
10. False.
11. Type I - only involves deficiency of red cell soluble enzyme. Type II - involves both the soluble and membrane bound forms of cytb5r. Cyanosis is also accompanied by severe mental retardation and neurological impairment.
12. (i) Direct oxidation of the haem iron, Fe^{2+} to Fe^{3+} , favoured by the absence of oxygen; (ii) indirect oxidation of the Fe^{2+} , a process that needs oxygenated haemoglobin, and is important in nitrite-induced production of methaemoglobinaemia; and (iii) a drug or chemical, such as the amino- and nitro-benzenes, may be metabolised to a derivative which is capable of initiating methaemoglobin formation.
13. Hb Ms can be identified by electrophoresis at Ph 7.1; differential UV spectrometry; mass spectrometry; and DNA sequencing.

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Motion:
Moved J Elliot, seconded K Beechey
That the Election of Officers be approved.

Carried

Awards

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

Qualified technical assistant awards

Clinical Biochemistry	Claire Cendana, Diagnostic Medlab
Medical Cytology	Dorothy McKane, Canterbury Health Laboratories
Haematology	Kaye McGeorge, Canterbury Health Laboratories
Histology	Jamie Anderson, Diagnostic Medlab
Immunology	Margaret Gibbs, Nelson Diagnostic Laboratory
Microbiology	Anna Newton, Hawkes Bay Hospital
Transfusion Science	Phillippa Stewart, Southland Hospital
Transfusion Science Blood Products	Nina Basson, New Zealand Blood Service
Mortuary Hygiene & Technique	Erin MacDonald, Rotorua Hospital
Phlebotomy	Lorraine Peterson, Nelson Hospital

Honoraria

Motion:
Moved J Broadbent, seconded G Crossley
That no honoraria be paid.

Carried

Auditor

Motion:
Moved R Hewett, seconded R Allen
That Hilson, Fagerlund and Keyse be appointed as the Institute's auditors.

Carried

General business

PPTC

-J Elliot thanked the Institute and Council for their support of the PPTC with respect to the donation made to this organisation. This donation helps run an external programme to 25 countries and the money that comes from the Institute is used for funding this programme. J Elliot acknowledged that PPTC does get a lot of laboratory technicians applying for courses that do not have any sponsorship. Asked that Council consider the money not paid to the IFMLS to go towards a scholarship for some of these people to attend the PPTC courses. J Elliot to formally write to Council with this request and Council to consider.

CPD for technicians

E Tibbs acknowledged that it was great to see the CPD for technicians is something that is going to happen.

K Taylor advised the meeting that another QMLT examination in Specimen Services will be available for next year. The Specimen Services syllabus and examination will be formalised at the next Council meeting and will be available in 2006.

R Hewett noted that the rebranding of the NZIMLS material and that Council is very happy with the look and all material will be printed in the same format.

Venue for the year 2006 Annual General Meeting

The 2006 AGM will be held in conjunction with the conference at Napier War Memorial, Napier, 21 - 25 August 2006.

Venue for the year 2007 Annual General Meeting

The 2007 AGM will be held in conjunction with the South Pacific Congress in Auckland at the Sky City Convention Centre August 2007.

Meeting closed at 8.15am

Pathogenesis of *Neisseria meningitidis*: a review of current understanding

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Abstract

Despite nearly 200 years of study and research we still do not know why *Neisseria meningitidis* causes severe disease with high morbidity and mortality, particularly epidemics lasting 5-10 years or longer. Much is now known about its transmission, the different clinical outcomes and their underlying pathophysiology and, in more recent years, using genetic techniques, new knowledge regarding the organism's virulence factors. However it would seem that the pathogenesis of *Neisseria meningitidis* remains a complex interaction between the organism and its ability to change and develop various virulence factors such as PilC, Opc, LOS and capsular phase switching, and the host immune response.

Key words: *Neisseria meningitidis*, pathogenesis, host immune response, virulence factors

Introduction

Meningococcal meningitis was first described nearly 200 years ago, and since then, study and research has proved that there is a complex interrelationship between the organisms and the host. Meningococcal disease occurs worldwide, being endemic in temperate climates, with sporadic cases and small clusters in winter and spring as well as causing major epidemics. Over the last 20 years serogroup B meningococci have been responsible for significant epidemics in Europe, Latin America, New Zealand and more recently in the United States (1,2,3).

The clinical picture of acute meningococcal disease has been well defined, about 90% have meningitis either without septicaemia or with septicaemia and generalized macular or petechial skin lesions. About 10% of patients develop fulminating sepsis characterized by a brief period of high fever, overwhelming septicaemia with endotoxic shock, disseminated intravascular coagulation (DIC), little or no evidence of meningitis and numerous, extensive purpuric and ecchymotic lesions on the extremities (purpura fulminans). Mortality in these patients is about 44% (4,5).

Some patients present with only septicaemia, with no meningeal involvement. A recent study seems to suggest that increasing numbers of patients are presenting with just septicaemia (6).

However, despite all that is known in regard to transmission, disease outcome, host immunity, etc., we still do not know exactly why one person may develop invasive disease and others do not, or why epidemics occur with specific serotypes. A significant problem hampering the study of meningococcal disease has been the fact that *Neisseria meningitidis* is a uniquely human pathogen, thus the lack of suitable animal models that can fully reflect the complex microenvironment seen during the disease processes. A number of animals such as mice, monkeys, rabbits and chicken embryos have been used to gain some significant insights (7,8,9). Consequently, it has been mainly in vitro cell and organ cultures studies, using human cells, cell lines or tissues that have significantly contributed to the understanding of the pathogenic processes (10,11,12). With new molecular technologies now available we are able to gain further knowledge of the mechanisms involved.

Pathogenesis of invasive disease involves at least 4 stages, and in the case of meningitis 7 stages. These are 1. exposure to a pathogenic strain; 2. attachment to epithelial cells of the naso-oropharyngeal mucosa; 3. penetration of the mucosal barrier; 4. survival in the blood stream, then in meningitis; 5. entry into the cerebrospinal fluid (CSF); 6. survival in the CSF; and 7. disease production in the meninges and brain. These processes are influenced by bacterial properties, environmental conditions, preceding or concomitant infections as well as the immune status of the patient.

The aim of this review is to look at the current understanding of the pathogenesis of *Neisseria meningitidis* in regards to these stages, focussing on the bacterial properties and host mechanisms involved (Fig 1).

Stages of pathogenesis

Exposure to *Neisseria meningitidis*

The human naso-pharyngeal mucosa is the natural reservoir of *Neisseria meningitidis*, carriage rate being 5%-10% and up to 50% in crowded conditions where people are brought together from different regions e.g. military barracks and boarding schools. The carriage rate depends both on the rate of acquisition and duration of carriage. Serogroups A, B and C are responsible for most meningococcal cases. Although serogroup A can cause outbreaks, it has mainly been responsible for epidemics in sub-Saharan Africa. In developed countries serogroups B and C are responsible for most meningococcal disease, in particular serogroup B has been responsible for protracted epidemics in various parts of the world. New Zealand is now in its 12th year of an epidemic with phenotype B:4:P1.4 (13) (refer Appendix 1).

Studies on meningococcal carriage have showed up to 50% of carrier strains were non-capsulated and until recently were considered non-pathogenic. It has now been shown that capsular expression is phase variable (14), and the loss of the capsule probably enhances the organism's ability to colonize the nasopharynx (15). The carriage of particular strains did not prevent colonization with a heterologous strain (15). Vogel et al describe a case where the serogroup changed from serogroup C to serogroup B within days of transmission as a result of the transfer of serogroup-specific genes (16). Colonization occurs both on the exterior surface of the mucosal wall and intra- or sub-epithelially with damage to the nasopharyngeal ciliated epithelium possibly being the first step in colonization.

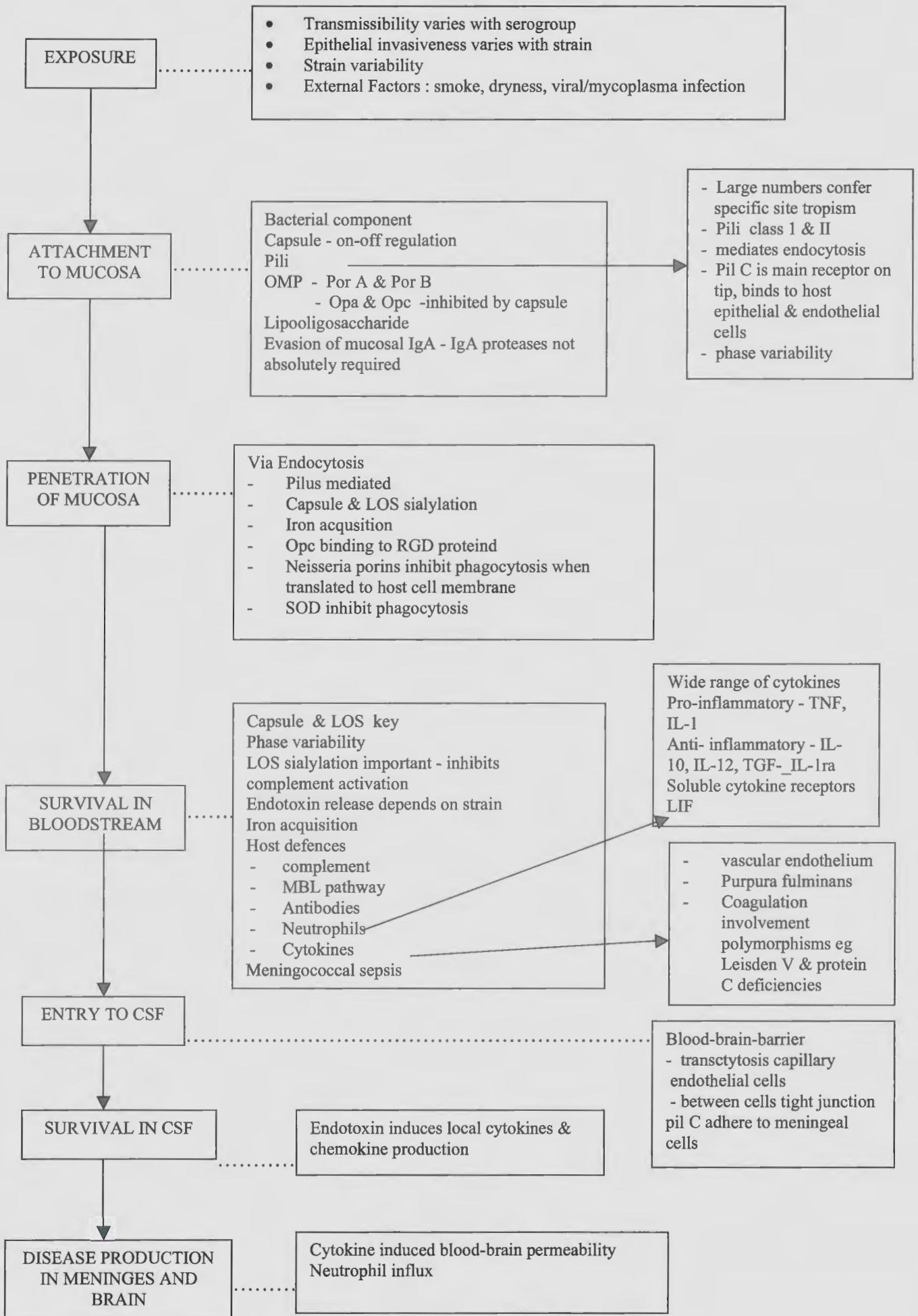
Physical damage by active or passive smoking (17), stressful events, prior upper respiratory tract infections due to viruses or Mycoplasma, and dry seasonal winds, e.g. in sub-Saharan Africa, are all predisposing risk factors for disease. Low socio-economic status is also a risk factor in that it is a marker for behavioural factors such as poor nutritional status, hygiene and overcrowding.

Vaccines have been developed and used with some success to control and prevent infection however there are a number of problems with them (18). (Refer Appendix 2)

Attachment to epithelial cells of the naso-oropharyngeal mucosa

The various surface constituents of the bacteria such as the capsule, pili,

Figure 1 Overview - Pathogenesis of *Neisseria meningitidis*



class 1 porin, lipo oligosaccharide (LOS) and class 5 outer membrane proteins (Opa & Opc) are known to play a part in the invasion process, but it has been hard to define the exact process as the bacteria seem to display strong intra- and inter-strain variations. Virulence in the pathogenic neisseriae appears to be modulated, in part, in a stochastic manner. Random high-frequency biphasic on-off or antigenic switching involving multiple gene families seems to control the progression of bacteria through the host (12). Specificity of attachment of pathogens also depends on the host cell receptor sites (19).

Capsule

Capsular polysaccharide is an important and adaptive virulence determinant. As mentioned earlier, the capsule undergoes phase variation. Capsule-deficient meningococci may be more capable of invasion of the nasopharynx whereas capsulated strains survive immune detection and killing in the serum (20). Capsule expression is known to be controlled by genetic on-off switch mechanisms. It has recently been shown that meningococci have the ability to switch polysaccharide capsules through genetic transformation (14,20).

Pili

Pili appear to be an important surface component mediating initial attachment of meningococci to human epithelial and endothelial cells, and nasopharyngeal mucosa (21). McGee et al found that meningococci with pili attached to nasopharyngeal cells in greater numbers than those without pili, and the attachment of piliated meningococci differed among epithelial cells of different sites whereas non-piliated meningococci attached to all cell types in equal but low numbers (22).

Pili belong to the type IV family of pilin, similar to the pili of many other Gram-negative pathogens. They are thought to be glycoproteins that may be modified by glycans (23). Meningococci produce two types of pili, class I and class II, which are morphologically indistinguishable but functionally similar. They consist of at least two distinct sub units, PilE that forms the pilus fibre, and PilC that is found at the tip of the pili. PilC has been identified as the adhesin important for binding to epithelial and endothelial cells whereas PilE and other pili proteins influence the recognition of the PilC receptor (21,24). Nassif and So's work indicates that organisms expressing high-adhesive bundles of pili and PilC1 are more likely to colonize the host (24).

Piliated meningococci attach to the microvilli of non-ciliated epithelial cells and interact with them and undergo endocytosis by the cells (25,26). Pili are not only subject to phase variation but also to antigenic/structural variations. As well as intragenomic recombination, intergenomic recombinations are common because Neisseriae readily take up DNA from other lysed Neisseriae. (23)

Outer membrane proteins (OMPs) - Class 1,2 and 3 OMPs - PorA and PorB

Porins are trimeric proteins that constitute pores that allow small solutes to diffuse through the outer membrane of the organisms. The neisserial porins are able to insert into the membranes of eukaryotic cells and have been shown in vitro to influence host neutrophil function thus inhibiting their phagocytosis. (27). They, like the other surface components, are subject to phase variation (28). This is particularly significant given PorA OMP is an important component of group B meningococcal vaccines.

Class 5 OMP - Opa and Opc

Opa and Opc protein play an important role in adherence as well as invasion in uncapsulated variants (24,29). A class of Opa proteins has been shown to bind to host receptors of the carcinoembryonic antigen

(CEA) gene family (29) thus facilitating phagocytosis and cytokine production (30).

Opc significantly increases adhesion and invasion. Different isolates vary in the amount of Opc protein produced, and the more Opc produced, the greater the adhesion of the strain (24). Binding to host receptors stimulates engulfment of the bacteria by host epithelial cells (30). While in encapsulated bacteria, Opa and Opc do not affect bacterial interaction with host cells (24).

LOS - endotoxin

LOS of meningococci is an amphipathic molecule consisting of a hydrophilic carbohydrate portion and a hydrophobic lipid A portion that attaches to the outer membrane. It is structurally different from that of enteric gram-negative bacteria (31). The oligosaccharide portion of LOS mimics the glycosphingolipid antigens found on the surfaces of human cells including erythrocytes (32). Structural analysis has shown that this oligosaccharide not only varies serotypically between stains, but that there is considerable microheterogeneity within a single strain (32). The significance of this has yet to be determined.

The absence of LOS seems to inhibit attachment to epithelial cells and therefore invasion of the host cells (31). A recent study using a mouse model showed a meningococcus mutant defective in LOS sialylation did not colonize (7).

LOS also is subject to antigenic variation and can be modified by sialylation thus inhibiting Opa and Opc mediated interactions with the host cells (24).

To effectively attach and invade, the bacteria must also evade the mucosal immunoglobulin A (IgA), actively secreted by the plasma cells. This is achieved by the production of IgA proteases (33) that cleave the proline hinge region of IgA rendering it non-functional, allowing the bacterium to attach to the epithelium (34,35). Mulks et al showed strains of meningococci may produce two distinct IgA proteases and that each isolate elaborates either one or the other but not both (36). These IgA proteases have also been shown to be potent inducers of tumour necrosis factor- α (TNF- α) in peripheral mononuclear cells (37). Recently type 2 IgA1 protease was found to reduce lysosomal protein levels in the host epithelial cell, thus promoting intracellular survival of the bacteria (38). While these proteases contribute significantly to virulence they are not a requirement for an invasive strain (39).

A recent study showed meningococcal carriage elicited a mucosal immune response as well as a cellular one (40). Invasive disease occurs when the patient lacks serum antibody, thus it is significant to note that those most susceptible to meningococcal disease, i.e. those under 2 years old, will not yet have developed any specific antibodies.

Penetration of the mucosal barrier

Following mucosal adherence and a period of adaptation and proliferation,

Meningococci are thought to pass through the mucosal epithelium via phagocytic vacuoles as a result of endocytosis (30,22,24,25,26). During this invasion process, bacterial factors as discussed above, involved in attachment, modulate the metabolism of the mucosal cell including the host phagocytic system. However all the processes have not yet been fully elucidated. A study by De Vries et al using monolayers of primary cultured nasopharyngeal cells, suggests that a concurrent phase switching of multiple surface constituents may be required to establish an invasive bacterial phenotype (12).

While pili play a part in adherence to cells, it is unclear whether they actually promote bacterial cell entry. However, they do determine host and tissue tropism as well as other important pathogenic functions such as DNA uptake and bacterial movement (23).

Cell surface-located sialic acids of the capsule and LOS are both important in mediating resistance to phagocytosis and complement-mediated killing via alternative pathway activation (41). It has been shown that the possession of a (α 2→8)-linked polysialic acid capsule resulted in decreased bacterial binding to human macrophages, most probably by interfering with phagosome-lysosome maturation (42). Since macrophages are able to kill meningococci, this is an important virulence mechanism (42). The ability to switch between a non-sialylated and a sialylated phenotype enables the meningococci to adhere and invade the mucosal epithelial cells (41).

The ability to acquire iron from the host is essential for survival. *Neisseriae* produce outer membrane proteins - transferrin, lactoferrin and haem binding proteins that bind host transferrin, lactoferrin and haem and remove iron from them (24).

Opc binds arginine-glycine aspartate (RGD)-containing serum proteins such as vitronectin that leads to cellular invasion through the subsequent binding of the RGD protein to its cognate integrin receptors. Further investigation is required as to how these binding functions may help the bacteria to survive (29,43).

PorA and PorB have been shown to translocate spontaneously as functional ion channels into plasma membranes of the host cells where they inhibit neutrophil attachment and ingestion as well as cell signalling. (27). This and other functions observed in vitro require further study.

Meningococci also produce superoxide dismutases (SOD) that catalyze the conversion of superoxide to hydrogen peroxide and oxygen. Copper and zinc co-factored SOD (Cu, Zn SOD) is found in the periplasm and it is thought that they protect meningococci from uptake and killing by various phagocytic cells (44,45).

Survival in the blood stream

Meningococci can survive and proliferate in the blood stream because of particular bacterial virulence factors or incompleteness of the host defence system (30).

Bacterial capsulation and LOS play an essential role in the pathogenesis of meningococcal septicaemia (46,47). Klein et al showed that bacterial capsulation and LOS structure protected against complement-mediated bacteriolysis and phagocytosis (46). With dendritic cells (DC), it has been shown the capsule mainly inhibits adherence, thus inhibiting phagocytosis, where as sialylation of LOS prevents phagocytosis and subsequent phagocytic killing (47).

Meningococcal LOS sialylation confers significant serum resistance partly by inhibiting the alternative complement pathway activation and opsonization as well as masking the epitopes recognised by human serum (46,48) This is essential for their survival in blood. Sialylation decreases adherence of bacteria to endothelial cells, therefore LOS immunotypes that cannot be sialylated appear to predominate in nasal carriage strains, whereas immunotypes that can be sialylated tend to be found in the blood.

Endotoxins also have a direct action on the properdin pathway and activation of the classical pathway may be activated as a result from combination of endotoxin with natural antibodies present in the sera (49)

During growth and lysis of meningococci, endotoxin is released in the form of vesicular outer membrane structures consisting of up to 50% LOS and OMPs, lipids and capsular polysaccharides (30,50). Endotoxin is the main mediator of the proinflammatory response and cytokine induction (47). Strains isolated from patients with septic shock liberate more endotoxin than do those with benign meningococcaemia. However it is not just the level of LOS, but also the production of certain cytokines and their levels that correlate to clinical outcome. The significant cytokines seem to be proinflammatory

TNF, interleukin-1 β (IL-1 β), IL-6, and anti-inflammatory cytokine IL-1 receptor antagonist (IL-1ra), IL-10, and transforming growth factor-beta (TGF- β) (51,52,53).

As mentioned earlier, the ability to acquire iron from the host is essential for survival. Meningococci have been shown to produce a haemoglobin receptor HmbR and a receptor HpuB for haemoglobin bound to haptoglobin, which are essential for the utilization of haemoglobin. Energy for the transport of iron from the host iron-binding proteins across the outer membrane into the periplasmic space is provided by outer membrane receptors tonB, exbB and exbI (54).

Host defences

Host defence after meningococcal invasion is determined by humoral and cellular responses belonging to the adaptive and innate immune systems. In meningococcal infection, the host inflammatory response is a major determinant of disease severity. The severity directly correlating with the degree of host inflammatory cell activation (46,55). The key components being complement, antibodies and neutrophil production (56,30).

Complement

The complement system plays a significant role in the host inflammatory response, being involved in a number of processes such as mediating chemotaxis of neutrophils, opsonization of organisms, inducing vasodilatation and enhancing vascular permeability (57).

Complement activation can occur through the classical pathway by antigen-antibody complexes, the alternate pathway by bacteria or LOS, and the mannose-binding lectin (MBL) pathway. As those who with meningococcal infection do not have antibodies, complement activation occurs by the alternate and MBL pathways.

The alternate pathway involves the interaction of factor B, factor D and properdin to generate C3b. Alternative pathway defects e.g. X-linked properdin deficiencies may predispose to overwhelming disease (58,59).

Inherited deficiencies in other complement components have been reported in association with severe or recurrent meningococcal disease (60,61,62,63). The significant components are those of the membrane-attack-complex (MAC). This complex is necessary for forming a lytic channel in *Neisseriae* as extra cellular lysis is a major mechanism in killing these organisms (64). It has been suggested that it is the insertion of the MAC into the bacteria and not the quantity formed on the surface that is important (65). However, due to the rarity of these deficiencies, they could only be only a small number of cases (30).

MBL pathway

MBL is a sugar-binding protein belonging to a family of calcium-dependent collagenous lectins, most of which are components of the innate immune system found in the serum of humans (49). On binding to its targets, MBL activates the complement system via MBL-associated serine proteases (MASP-1 and MASP-2. MASP-2 cleaves C4 and C2 to generate a C3 convertase independently of immunoglobulin (66,67). MBL may also interact directly with phagocytic cells to regulate their function. Serum levels of MBL are genetically determined via 3 structural gene mutations and 3 promotor region polymorphisms (68).

It has been reported that some genetically determined variants might predispose individuals to meningococcal infection (69,68,49,70). The expression of capsular polysaccharide has been shown to decrease the binding of MBL to *N meningitidis* serogroup B as does the structure and composition of bacterial endotoxins (67).

However it seems the lack of the sialic acid acceptor site or removal of sialic acid rather than encapsulation, which is important in

determining MBL binding to bacteria.

Antibodies

Studies have shown that susceptibility to systemic meningococcal disease is related to a deficiency of humoral antibodies to meningococci. There is an inverse correlation between serum bactericidal activity and age-related incidence of meningococcal infection with the risk of disease decreasing with increasing age. At least 40-50% of cases occur in children under 5 years, most under one year old (71,72,73). Antibodies can be acquired either by passive immunization by transplacental passage of immunoglobulins or active sensitization due to the meningococcal carrier state. Goldschneider et al's study suggested that natural immunity to meningococcal disease is initiated, reinforced, and broadened by intermittent carriage of different strains of meningococci throughout life (74). Epidemics tend to show a shift in the number of cases towards older age groups, in part due to them having no previous exposure to that particular strain (72,71). It is interesting to note that in the New Zealand epidemic, there has not been the shift to the older age groups, with most cases still occurring in those under 5 years of age - in 2001 44% were under 5 years (71).

The bacterial activity of normal serum to meningococci can be attributed largely to γ M globulin. Hobbs et al reported low γ M globulin in patients with fulminating meningococcal septicaemia patients, suggesting these were most likely inherited deficiencies (75). IgG₂ subclass deficiency has also been reported as being a predisposing factor for recurrent meningococcal disease (76). Circulating IgA antibody to *N meningitidis* capable of blocking the complement-mediated bacteriolytic activity of IgG and IgM may also make people more susceptible (77). However the role of blocking IgA antibody is still uncertain (30, 73).

Cellular immunity defects may also predispose an individual to disease. Immunosuppressive drugs and autoimmune disease are reported predisposing factors. Splenectomy has also been well defined as a risk factor for overwhelming disease with encapsulated bacteria (30).

Neutrophils

Neutrophils are natural effector cells mediating antimicrobial defence by the production of a wide range of cytokines (78). Opsonic and non-opsonic phagocytosis by neutrophils, as well as complement-mediated bactericidal activity in serum, are important in the host defence against *Neisseria meningitidis* (56,30).

Neutrophils are both friend and foe. They are essential for the eradication of pathogens, but the oxidants and proteases they release as a result of activation and adhesion are also toxic to host tissue (79,46).

Cytokines

Endotoxins, as discussed earlier, induce the production of a wide range of cytokine. Pro-inflammatory cytokines, particularly TNF and IL-1 are essential for adequate host defence (52,53,80,81). Their functions include inducing chemokines and adhesion molecules as well as stimulating phagocytosis and tissue repair. In high concentrations, TNF- α can cause wasting of muscle, septic shock and death (53). The pro-inflammatory effects of IL-1 are mediated by IL-1 α that is mainly cell-bound and IL-1 β , which is released. IL-1 β is considered as an important mediator in the complex pathogenesis of septic shock and bacterial meningitis (81).

Likewise anti-inflammatory cytokines and chemokines, eg.IL-10, IL-12, TGF- β , and IL-1ra are also produced (52,53). These anti-inflammatory cytokines inhibit the host immune response. The anti-inflammatory response also includes soluble cytokine receptors which

down regulate their specific cytokine e.g. TNF activity is down regulated by soluble TNF receptor (sTNFR) (80). Principle source for cytokines are blood monocytes, tissue macrophages (52) as well as specialised antigen-presenting cells such as dendritic cells (82,47).

Another recently described cytokine, cytokine leukaemia inhibitory factor (LIF), can be produced by a number of cells, including fibroblasts, monocytes, macrophages, T lymphocytes, and endothelial cells (83). It stimulates the acute-phase response, primes the immune system and phagocytic cells. LIF synthesis can be induced by cytokines TNF, IL-1 and endotoxin. Along with other acute-phase cytokines it contributes to pathogenesis of septic shock (83).

Meningococcal sepsis

Sepsis occurs when the immune system is severely compromised and unable to eradicate pathogens (79). Septic shock results in marked inflammatory response, leading to capillary leakage, DIC, vascular injury, and, ultimately death. Complement plays a key role in that it contributes to the host's defence but may also contribute to tissue damage and severe complications (57).

In the early stage of disease both pro and anti-inflammatory cytokines are increased, probably as a result of generalized activation (84). However, in severe shock, although both pro and anti-inflammatory cytokines levels are raised, the levels of anti-inflammatory are much higher (85). van Dissel et al found, by measuring TNF- α and IL-10 levels in febrile patients, that the ratio of IL-10 (anti-inflammatory) to TNF- α nearly doubled in those patients who died.

A major target for inflammatory response in meningococcal disease is the vascular endothelium. Recent studies have shown that meningococci have the capacity to bind endothelial cells in a receptor-ligand-specific fashion and that this bacterium-endothelium contact may be critical in mediating the vascular damage seen in this disease (86). Dixon et al demonstrated that LOS was important in determining the pattern of vascular endothelial adhesion molecule expression and that even subtle changes in the LOS structure influenced the host inflammatory response (86).

Purpura fulminans is a syndrome characterised by intravascular thrombosis and haemorrhagic infarction of skin, limbs and digits. The clinical presentation of skin haemorrhages is characterized by endothelial damage with haemorrhage around and microthrombi within the small vessels (87). The lesions are a reflection of the endotoxin and cytokine vasculitis mediated by the up regulation of adhesion molecules on endothelium and degranulating activated neutrophils.

Recently Harrison et al demonstrated that meningococci that reached the skin invade the deep tissues, forming micro colonies that expressed three key virulence factors - capsule, PorA, and pilin (88) with phase variation occurring. These findings confirm in vitro studies regarding their importance in pathogenesis of meningococcal disease.

Fulminant meningococcal sepsis also involves the coagulation systems, which is different to meningitis where coagulation factors are not involved. It has been shown that deficiency of Protein C, a regulator involved in the coagulation, anticoagulation and fibrinolytic systems, leads to extensive DIC and necrosis (89,90). Recent work by Faust et al has shown that the endothelial protein C activation pathways are impaired (91).

The Factor V Leiden mutation that has been associated with thrombosis may be a risk factor for developing fulminant meningococcal disease. However, it is most likely a combination of factors are responsible for the dysregulation of the coagulation cascade in meningococcal disease, including low levels of antithrombin III, protein C and Protein S, up-regulation of tissue factor expression on monocytes, and high plasminogen activator inhibitor 1 (92,93).

Entry into the cerebrospinal fluid

Compared to other extracellular pathogens, meningococci have a tendency to invade the meninges. From the blood, meningococci can gain access to the meninges by crossing the blood-brain-barrier (BBB). Entry is gained either by transcytosis through capillary endothelial cells or by passage between the cells after disruption of the tight junction (24). Alternatively, the choroidal plexuses, the major site of CSF synthesis, is another possible route into the CSF. The choroidal plexuses are situated in the ventricles of the brain as leaf-like structures with a central core of blood vessels covered either side by epithelium. To cross the blood-brain barrier at this site would involve crossing the epithelial layer (24). Dural trauma has also been implicated as a risk factor for meningitis (94).

Neisseria meningitidis shows a specific predilection for binding to the leptomeninges and meningeal blood vessels in human brain but not to the cerebral cortex (95). While the mechanisms by which meningococci gain access to the subarachnoid space and the molecular bases of the interactions between the meningococci and cells of the leptomeninges are poorly understood, *in vitro* studies showed the major ligand that mediated adherence of meningococci to both meningoma cells and leptomeninges appeared to be the pili (95). Pron et al showed PilC played a significant role in the crossing of the BBB, most likely through pilus-mediated adhesion (96).

Survival in the CSF

Having crossed the blood-brain barrier and into the subarachnoid space, where the main humoral and cellular host defences are absent, meningococci can proliferate uncontrolled (30). Endotoxins induces proinflammatory cytokines, e.g. TNF, IL-1, IL-6, platelet activating factor, and anti-inflammatory cytokines, e.g. IL-1Ra, IL-10 (95). Increased levels of chemokine IL-8 (CXCL8), growth-related oncogene alpha (CXCL1), monocyte chemoattractant protein 1 (MCP-1), and macrophage inflammatory protein 1a (MIP-1 α) and (MIP-1 β) are also seen (97). Cytokine production precedes to the rapid influx of neutrophils and later, monocytes and T cells (53,9).

Wells et al's study, using human meningotheial cells, showed that these cytokines and chemokines were most probably from the cells of the meninges (97). These cytokines are produced by the cells of the meninges and their function is confined to the local inflammatory reaction in the CSF compartment. (97,9).

Disease production in the meninges and brain

Studies have shown that there are separate compartmentalised intravascular & intracranial inflammatory responses to infection (82,9). Cytokines IL-1 and TNF enhance the permeability of the blood-brain barrier and promote the influx of neutrophils by up regulation of adherence molecules. The subsequent release of neutrophil products contributes to the development of clinically overt meningitis (30). Chemo attractant and activator, N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP) that is produced during bacterial cell lysis also contributes to the infiltration of granulocytes (53).

The anti inflammatory cytokines IL-10 and transforming growth factor- β (TGF- β) which control the pro-inflammatory cytokines may in certain situations impair host defences (53)

Other bacterial considerations

As discussed earlier, the bacterial constituents important in pathogenesis show strong intra- and inter-strain variations. These phase variations are associated with reversible mutations at individual loci termed "contingency loci" (98,99). Loci have been identified associated with

various function-related gene groups such as evasins, LOS biosynthesis, adhesins, iron acquisition and restriction-modification systems (98). Many mobile DNA elements transpose from one chromosomal location to another (100). They include insertion sequences, transposons, phages and pathogenicity islands (100,101). In pathogenic bacteria it is thought that some of these elements may be responsible for the exchange of genetic material coding for virulence traits (100). For example, Kahler et al described an exchangeable DNA island *exl* that consists of at least 3 distinct cassettes, one of which encodes the Hb receptor (101).

RTX-cytotoxin- related proteins, two Fe-regulated proteins FrpA and FrpC, have recently been described associated with and secreted by the outer membrane (102,103,104). Although the functions of these meningococcal RTX-like proteins are unknown, given they are related to other bacterial cytotoxins it is possible they may contribute to the severity of disease (104,105). In a recent study they did not appear to have any toxic affect on infant mice, however, other roles in disease process could not be excluded (106).

Dendritic cells are specialized antigen-presenting cells found as a trace population in most tissues, when activated, start to capture and process antigens. Dendritic cells appear to play a crucial role both in the initiation and modulation of specific immune responses as well determining the type of response (82, 107). Further study into dendritic cell-bacterial interactions may be useful in finding ways to control the disease severity.

Bacteria have mechanisms known as quorum sensing (108) that co-ordinate gene expression in response to population density (109). These involve the production and detection of signalling molecules (auto-inducers), which modulate critical functions including virulence factor production. Winzer et al describe the presence of a *luxS* gene that is required for the production of a signal molecule called auto-inducer-2 (AI2) (109). Their study showed this gene contributed to the virulence of the organism.

Conclusions

Because meningococci only interact with human cells, our knowledge of their colonization and pathogenesis has been derived largely from sub-optimal study models.

However, studies have shown that meningococci have greater genetic diversity than most other human bacterial pathogens (30) and display strong intra and inter strain variation in their surface constituents (12,110). Pathogenic meningococci are able to regulate their virulence at many levels (22) and it has been shown that bacterial cell interactions are essential not only for pathogenesis, but also for other aspects of their survival and dissemination (111,99,112,113).

The capsule, LOS, PilC1, Opc and certain Opa are important bacterial components in the initial adhesion and invasion, and then once in the cells other virulence factors also play a part in survival and induction of the disease process (24). Highly significant in pathogenesis is the degree to which pathogenic meningococci regulate their virulence factors throughout the different stages of the disease process (98). Expression of these factors are regulated either by phase/antigenic switching, e.g. pili and LOS, or environmental stimuli e.g. they undergo a variety of genetic modifications of their outer membrane proteins, particularly in respect to utilising host transferrin in an iron-depleted environment (101,98,100).

Using molecular typing methods it seems that invasive disease and significant epidemics outbreaks are caused by a few pathogenic meningococcal clones spreading around the world (30,15). It is still largely unclear why only some regions are affected, and compared with the prevalence of meningococcal carriage and transmission, invasive disease is relatively uncommon. However, it is known that certain host

and environmental factors do increase the risk of invasive disease e.g. prior upper respiratory tract infection or overcrowded conditions.

The host immune response to meningococci also plays an important role in determining disease occurrence and severity. As van Deuren et al noted, invasive disease seems to occur only in patients lacking specific bactericidal or opsonizing antibodies (30). Carriage of meningococci may elicit an antibody response for a specific strain itself and which can cross-react with heterologous strains (40). However, colonization does not protect against re colonization with that same strain, heterologous strains, or always protect against invasive disease (40). In contrast, the immune response elicited by invasive disease does cross protect against recurrence of disease (74). Since *Neisseria meningitidis* continues to be the major cause of bacterial meningitis and septicaemia in children, with high associated morbidity and mortality, it is important to develop effective vaccines to prevent meningococcal disease.

It is clear that both bacterial and host factors play important roles in pathogenesis, however there still remains many unanswered questions with regards to all the complex interrelationship processes involved. It has been suggested that certain genetic genes are required for bacterial transmission and pathogenesis (113).

Little is yet known about the regulatory networks responsible for the expression of *Neisseria meningitidis* virulence factors. With the now available technology for genetic analysis, there has been increased knowledge regarding the complexities involved in meningococcal disease. For example, Dietrich et al found that when bacteria adhered to human epithelial cells, 72 genes ORFs were differentially regulated and to brain endothelial cells 48 ORFs (112).

It has been shown that genetic factors influence an individual's susceptibility to meningococcal disease (79). Genetic alterations that have been identified include polymorphisms in TNF receptors, IL-1 receptors, Fcγ receptors and TLRs, all of which influence cytokine production and therefore the inflammatory response (114,115,79).

Further understanding of the processes involved in pathogenesis, particularly the regulatory pathways is important for future strategies in the prevention of meningococcal disease.

Appendix 1

Epidemiology of meningococcal disease

Meningococci are divided into 13 different serogroups using their capsular polysaccharides: A, B, C, D, W-135, X, Y, Z, Z' (usually referred to as 29E), H, I, K, and L (116). There appears to be a difference in the transmissibility of the different serogroups with most disease world-wide caused by five serogroups, A, B, C, W-135, and Y, in particular serogroups A, B and C. Serogroups A and C, which tend to cause outbreaks in closed communities, are easily transmitted whereas certain epidemic strains of serogroup B have low transmissibility but high virulence (117). Serogroup A and C epidemics usually resolve in 1 to 3 years however serogroup B outbreaks begin slowly and may persist for decades, with a significant number of the cases occurring in children under 5 years old (118). Since 1974 there have been significant serogroup B epidemics in Norway, England, Latin America and more recently in the 1990's in New Zealand, Belgium and the United States (1,13).

There are currently 20 serotypes defined based on Class 2 and 3 Outer membrane proteins (OMP) and at least 10 types based on Class 1 OMPs (116). Thus B:4:P1.7b,4 (the current NZ epidemic strain) represents serogroup B, serotype 4 and serosubtype P1.7b,4. (119).

It is also important to note that most cases of meningococcal disease are caused by a small number of genetically defined clonal groups and it is these specific clonal types which seem to cause protracted epidemics in the world. Multilocus enzyme electrophoresis testing from these serogroup B outbreaks show that they mainly belong to 4

electrophoretic types (ET). ET-5 complex, cluster A4, lineage III and ET-37. ET-5 complex being the cause of the outbreaks in Norway, Cuba, and more recently United States (72), however they do not necessarily share some of the OMPs (118).

ET-37 complex containing more than 50 different ET's is responsible for most outbreaks and sporadic cases in United States (120).

The New Zealand strain B:4:P1.7b.4 belongs to lineage III (ET-24 and ET-25) and is indistinguishable from those that caused outbreaks in the Netherlands in the 1980's and Belgium in the 1990's (121). In 2001 the rate of meningococcal cases was 17.4 per 100,000 population compared to pre epidemic rate of 1.5 per 100,000 with a case fatality rate of 4.0%. As in other years the rates were highest in the <1 year of age and 1-4 year age group with rates of 205.0 and 81.4 per 100,000(71,119). Since mid 1990's this clonal complex has also been reported in United Kingdom, Belgium and Chile (30).

Appendix 2

Meningococcal vaccines

Effective vaccines have been developed for serogroups A, C, Y and W-135 using their capsular polysaccharides and have been used in controlling epidemics caused by these serogroups since the 1970's. However, they are not useful for routine vaccination as they only give short-term cover, have low efficacy in children 2 to 10 years, and are ineffective in children under 2 years (18).

Recently, conjugate meningococcal vaccines have been developed where the polysaccharides have been conjugated to carrier proteins. These conjugated vaccines have been shown to be both more immunogenic in infants and induce immunologic memory, making it possible to use then in vaccination programmes (122). The United Kingdom implemented routine immunisation with a conjugate serogroup C meningococcal vaccine in 2000.

Because the serogroup B capsule is poorly immunogenic, vaccines using the PorA component of the outer membrane protein (OMP) have been developed with varying success (1,2,3). This is due to the PorA variable region being highly variable and that a minor change can alter immune recognition (123). While most cases of serogroup B meningococcal disease have been caused by a small number of genetically defined clonal groups, e.g. in the last 20 years, electrophoretic type (ET) -5 has been the cause of the outbreaks in northern Europe and Latin America as well as New Zealand and the US Pacific Northwest (1,13,124), they do show significant strain variations. Results from the study by Tappero et al showed vaccines made from the class 1 OMPs from the epidemic strain are more effective than those from other strains in protecting that particular population (3). New Zealand has developed a strain B:4:P1.7b.4 PorA specific vesicle vaccine that is currently being trialled (125).

A number of other non capsular meningococcal antigens have also been studied for possible inclusion in vaccines: transferrin binding proteins TbpA and TbpB can elicit a strong immune response and could be useful being included in a vaccine (126,127), neisserial surface protein A (NspA) (128,129), as well as Opa and Opc.

The ability of *N meningitidis* to "switch capsules" by genetic transformation introduces another challenge to find a suitable vaccine (14).

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OBITUARY - Hugh Grovenor Bloore

3 March 1918 - 10 September 2005

They looked a rather strange group of 'obstetricians' as with two senior medical consultants they contemplated the new infant just produced. Conception had taken place quite some time earlier - the gestational period considerably longer than the classic three trimesters. Now the delivery had occurred and the child duly named and registered - *The New Zealand Association of Bacteriologists*.

The godparents (*The New Zealand Society of Pathologists* (represented by Thos Pullar and Dennis Stewart) looked on proudly if somewhat indulgently. The obstetricians also looked at the fledgling child and secretly dreamed of the day when the umbilical cord might dare be cut. The youngest of that group - men such as Whillans, Buxton, Carruthers, Mckinley - was the man from Blenheim - **Hugh Bloore**.

A man passionate about his position of Bacteriologist; passionate that the profession should become independent; passionate that those who came into that profession would be properly and completely trained. He was prepared to do more than talk about it - he got involved in the Association, and particularly in the development of the examination system. That *Association* finally morphed of course into our present *Institute*, so in a very real sense Hugh was one of our 'founding fathers'.

Hugh Bloore was born in Gisborne but during depression days the family moved to Wellington. Hugh started work in the laboratory at Wellington Hospital and over seven years achieved his BSc at Victoria University.

In 1944 Dr Mercer invited him to establish the laboratory at Wairau Hospital in Blenheim where he spent the rest of his working life. When he shifted to Wairau Hospital and turned out to work in his white laboratory coat it was rather to the chagrin of the hospital doctor who, at that stage, was the only person qualified to wear a white coat.

Hugh worked in the laboratory during times of great change and it was to his credit that Blenheim, despite its size, was never far behind the leaders and ahead of many larger laboratories. Hugh's drive and a supportive and enthusiastic Board guaranteed that. He had a great feel for instruments - often with a screwdriver or socket in hand - which was generally in his favour but on occasions led him into some trouble! He was the consummate Kiwi DIY handyman with wide interests including woodwork, radio and photography, as well as being quite passionate about the outdoors - cycling and tramping large areas of the South Island over the years.

His hospital duties ranged beyond the laboratory walls and there can be few if any other technical staff who have had their photo in the local newspaper performing replacement transfusion on a neonate. In fact Hugh's clinical acumen was quite outstanding and in early days he was invited on ward rounds by the medical staff. Hugh worked until 1985, having worked for 48 years in a medical laboratory.

As indicated in the opening paragraphs of this tribute, Hugh developed an early and abiding interest in the profession through

its representative body and from 1954 to 1966 he served on Council as Councillor, Vice President and President. During that time he invested huge amounts of time and expertise into the growing of the examination system. Following his time on Council he was made a Life Member of the Institute, an honour he never esteemed lightly, and indeed not long before his death he reminded someone about it.

This scribe's own recollections of Hugh at Council meetings and Conferences is of a man of prodigious energy, abounding humour and execrable puns, whose knowledge of the latest in scientific endeavour was quite outstanding (he went on reading science journals to the end).

Hugh's wife (Ethne) died in 2000, but he is survived by his son, Chris, and two daughters Judy and Liz and five grandchildren.

As an Institute we join the family in celebrating with gratitude the very full life of Hugh Bloore, and equally we offer our sincere condolences to them on their loss, and give them our sincere thanks for sharing Hugh with us.

Hugh's funeral was held at St Andrews Presbyterian Church in Blenheim on September 14th. A Guard of Honour from the laboratory and a large congregation drawn from friends and community testified to Hugh's diverse interests and the regard in which he was held.

Desmond Philip

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