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1

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TH Pullar Memorial Address
What makes athletes super? Medical laboratory science has many answers

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It is a great privilege to be invited to give the T H Pullar address to this the year 2001 Annual Conference of the New Zealand Institute of Medical Laboratory Science. When I received the invitation in the mail it was only natural that I wondered why I was chosen to give this address. The grey hair certainly tells you that I have been around for a few years and all my working life has been in this profession. I have also been involved with a number of organisations associated with medical laboratory work, especially in the educational area. The invitation, however, said that I was known to have an interest in cycling and that this interest was in keeping with the theme of the conference.

So I had a dilemma, Should I follow previous speakers and talk about our profession, a state of the nation, where are we going, type speech, or was I being given license to take a different approach. I agonised over this question, as it is certainly a real honour to be asked to give this address in the memory of Doctor Pullar. Tos, as he was known, did so much for medical laboratory scientists in the infancy of our profession and it is important to remember him in a fitting manner. I know that there is any number of you who are better qualified than me to talk about the state of the profession. So, I decided, after reading the invitation numerous times to obtain reassurance, that perhaps I could branch out and present an interesting insight into the world of cycling at the top level.

One of the really interesting things about cycling and sport in general is that it is all about the human body, a fit human body. While we in our medical laboratory profession are largely dealing with the pathology of sick patients, a great deal of research and study has gone into top sporting athletes who are in prime physical condition. Professionalism and the huge monetary business that is an integral part of sport today, have stimulated a huge interest in the search for a greater understanding of how the human body performs and how it can be made to perform better.

Search through PubMed on the Internet and you will find many research papers directed towards the physiological changes that occur in super athletes, the factors that enhance an athletes performance, and factors that may cause poor performance. There are many journals specifically publishing papers on these subjects. Unfortunately, a great deal of time, effort and money is also spent on preventing athletes from enhancing their performance by illegal means. It seems that wherever large sums of money are involved, there is often an overwhelming temptation for athletes to cheat. Cycling certainly attracts more than its fair share of publicity in this regard.

Today I would like to take you through a stage of the 2001 Tour de France. Along the way, I will discuss some of the physiological and nutritional demands that the cyclists are subjected to in this gruelling 3600km, 20 stage race. The Tour de France is one of three major tours, the Giro d'Italia and the Vuelta Espana being the others. There are many other one-day races and shorter tours on the cycling calendar, but the major tours place special demands on the cyclists to the extent that most riders can only ride one or two a year. Training and preparation for one of these tours takes five to six months of specific activity with the sole aim of starting the Tour just below peak fitness. Winning a major tour is all about using energy wisely and to maximum effect, conserving that energy as much as possible and to start each day as fully recovered as possible.

Not all cyclists have the ability to win a long tour such as the Tour de France. The 20 stages are made up of a mixture of terrains and disciplines and it is the cyclist with the lowest overall time for all 20 stages whom wins. The first week is dominated by flat stages designed to allow the riders to adapt to the demands of daily racing. Cyclists who are very good at sprinting take their opportunities to notch up a win before the peloton reaches the mountains. One or two stages are devoted to time trialing where the riders must compete as individuals without the aid of team members or the wind drafting effects of the collective group. Finally there are the mountain stages, where many valuable minutes are won or lost.

To be a winner you have to be a good time trialist and mountain climber. Each of the twenty teams competing has nine members with only one or two capable of winning the overall yellow jersey. The rest of the team is there to support and protect their leader in his efforts to gain time on the field. Teams, which do not have an overall contender, will aim for stage wins and some will merely aim to provide their sponsors with as much television exposure as possible.

Boundless natural ability, months of training, thousands of dollars worth of equipment and back up are essential for a tour contender. Extraordinary mental strength is crucial. But even when all the factors are lined up, a simple error of judgement can cost minutes and even the yellow jersey.

The food and drink that fuels the peloton over the climbs and along the flats is the most vital element of their performance. If a rider misses out on even a handful of calories the result can be devastating. Anyone who saw the Tour last year may remember Lance Armstrong struggling badly on the climb to Morzine after missing a feed. It has been shown that repeated bouts of high-intensity activity can result in depletion of intracellular glycogen (1). Since glycogen is the fundamental fuel used to sustain both glycolysis and oxidative phosphorylation, fatigue becomes readily apparent as cellular resources are exhausted. It is sometimes called hitting the wall or getting the bonk.

Conversely, studies show that elevated starting muscle glycogen content will postpone fatigue by approximately 20% in an endurance event (2,3). This can be achieved by what is known as carbo-loading. A few days prior to an event, glycogen levels are allowed to run down by reducing carbohydrate intake and maintaining training effort. Then just before the event carbohydrate intake is increased markedly. The body stores as much energy as it possibly can, resulting in higher than normal levels of glycogen.

In an event such as the Tour with hard racing every day that is not possible so it is vitally important for the riders to maintain their glycogen stores day after day. The daily intake of carbohydrates is geared to provide, as many calories required for exercise as possible and to replace the glycogen stores if they have been reduced. While this graph shows the effects of high and low carbohydrate diets on 2 hour training bouts the same principle applies to daily racing.

To illustrate the calorific volume of a rider's daily intake let's convert to that well known universal standard - the cheeseburger weighing in at around 320 calories. Nine thousand calories is the amount of energy required in a mountainous Tour stage such as the one we will look at. Spread out across the day the feeding pattern is something like this: breakfast - 4 cheeseburgers; elevenses - 4 cheeseburgers; lunch - 6 cheeseburgers; afternoon tea - 4 cheeseburgers; dinner - 7 cheeseburgers; bedtime snack - 3 cheeseburgers. We now have to reshuffle the burgers to fit the riders eating patterns. For breakfast it's 5 cheeseburgers, a couple to snack on during the day, 14 for dinner and a mere half a dozen during the evening; a grand total of 28 in one day. I don't expect you will want fries.

Back to reality and what do they really eat? Breakfast is eaten three hours before the stage start to allow for complete digestion and to get the maximum benefit. Typically it is made up of the conventional Continental ingredients: rolls, ham, cereal, toast, yoghurt and fruit, plus a plate of plain pasta. Dinner is equally straight forward; two big plates of rice or pasta, lean chicken, occasionally a little lean red meat to restore iron levels, or possibly some fish providing it's fresh. Food poisoning during a tour would be a disaster.

That takes care of replenishing the body stores, now the riders can indulge in a little dessert. Ice cream is off the menu though - too much fat - but an occasional glass of alcohol is okay especially to celebrate a win. Speaking from personal experience, beer, which is alkaline, tends to be easier on the stomach than wine. You might think that even this real food would take a bit of consuming but you have to remember that the competitors are burning up 9000 calories a day and that means a ravenous appetite. Cyclists have a saying when asked what they want to eat. The answer is "See food". - "See food and eat it." Even after consuming all this food it is still insufficient for daily requirements of strenuous exercise. The difficult part of the fuelling process is to take on board enough energy during the stage itself to avoid depleting the muscle glycogen. Most of this energy comes from the drink bottle otherwise known as the bidon, or from the squeezy gel sachet.

Water, the most basic requirement, is essential during one of these hot stages. The riders will need about 10 litres a day as they can lose up to 7 litres as sweat - towards the end of the stage when the exercise intensity is greatest, fluid loss will be 2 litres an hour. The general rule of thumb for water replacement is one litre per hour. The two main by-products of intense and prolonged oxidative metabolism that can limit performance are the accumulation of lactic acid and elevated body heat. When the hypothalamus senses an increase in core body temperature above 37°C, the hot blood at the core moves to the skin where perspiration acts as a cooling mechanism. The cooler blood returns to the vital organs to lower the core temperature.

Dehydration during exercise promotes hyperthermia by reducing skin blood flow, leading to reduced sweating rate and thus heat dissipation. The combination of dehydration and hyperthermia during exercise causes large reductions in cardiac output and blood flow to the muscles. This has a large potential to impair endurance performance.

In addition to the cooling effect provided by an adequate supply of water, a study in Australia by Hargreaves demonstrated that when fluid was ingested at sufficient rates to prevent dehydration, heart rate was lower throughout exercise and muscle glycogen utilisation was reduced by 16% (4). Graph 2 shows that a water loss of 4% to 6% of body weight severely effect performance. Ryan and colleagues found that absorption of water is enhanced when carbohydrate is added to a concentration of 5% (5). In their study they tested water, glucose, glucose polymer and glucose polymer plus fructose. The glucose polymer plus fructose solution showed the lowest change in plasma volume during laboratory trials, indicating more rapid absorption of this fluid mix. Most commercial drink formulas use sucrose rather than fructose. Whether there is a scientific reason for that I don't know.

The squeezy gels, mentioned earlier, are a fairly recent innovation. They are a high concentration (approximately 50%) mixture of carbohydrates in the form of simple sugars that are contained in little squeezy bottles or plastic tear top sachets. They are usually consumed towards the end of the stage, as they require a large amount of water to be taken at the same time. Water is often more refreshing and readily available late in a race. Most riders during the races consume solid food in small amounts. These days, commercial energy bars have largely replaced sandwiches, rice cakes and other homemade goodies. Most energy bars look like a piece of dirt the car has run over, and definitely cannot be eaten with dentures, but they taste good.

The day is Tuesday July 17th 2001, Stage 10 of 209km with 61km of climbing. It is the first day of real mountain climbs in the French Alps and Figure 3 is what the profile of the course looks like. One small climb followed by three Haut Category climbs. All climbs are categorised and Haut Category is for the monsters. The finish is at the ski resort of L'Alp d'Huez. The stage starts at 10.30 am in Aix-Les-Bains so that means breakfast at about 7am. A bit of time to kill before the start but there is gear to pack up and load onto the team transporter. Most riders will relax on their beds keeping weight off their legs as much as possible. Robert Miller, a successful Scottish cyclist and colourful character provided this advice. Never stand when you can sit. Never sit when you can lie down. They will also be constantly sipping on drink bottles to ensure complete hydration. At the start line the pockets are full of squeezy gels, energy bars and the two bidons are topped up ready to go.

At 40km there is a category 3 climb to 950m above sea level but the climb itself is only about 400m. A short and sharp 2km climb. There will be little action at this stage as most riders attempt to conserve energy for the three Haut Category climbs to come. Along the flats to Albertville and at 87km the peloton passes through the one and only feed station for the day at the foot of the Col de Madelaine. In days gone by the feed stations could be found in all sorts of idyllic spots and it was not a problem to take time out for a civilised luncheon. The modern race rider is fed swooping past a helper with his arm outstretched, ready to snare the strap of the feed bag. Solid food such as well as gel sachets are handed to the riders in a bag, which is then hung round the neck while they retrieve the goodies. It's vital not to miss this feed, as there won't be another chance.

By this stage the riders have been on the road for about 23/4 hours and they will have drunk nearly 3 litres of carbo drink and eaten the odd high-energy bar to avoid delving into their body stores. The temperature in the enclosed valleys is very warm through the middle of the day so these buckets of water are very welcome. Drink bottles can be taken from team cars at any time but it usually requires one of the domestiques to drop back to the following convoy of cars, load up as many as possible before riding back up to the peloton to pass the bottles around the team members. Once they enter the mountains, it won't take long for the cyclists to end up in small groups strung out along the road and it becomes much more difficult to get supplies from the team cars.

Now the Madelaine is the first of the really big climbs rising to 2000m from about 400, a 25km climb. It's important, at this stage of the race, to keep the heart rate just below the Anaerobic Threshold - the point at which aerobic glycolysis changes to anaerobic glycolysis. This threshold is measured as the maximum heart rate at which lactic acid levels begin to rise above steady state. Go beyond the Anaerobic Threshold for too long and lactic acid builds up and power output drops. There is a risk of muscle damage and cramping may set in later on in the stage. In 1994 a Norwegian by the name of Medbo published a paper that showed that 60% of the glycogen lost during intense exercise reappeared as lactate within the working muscle (6).

Endurance training is designed to increase exercise intensity while still maintaining aerobic glycolysis and thus avoid lactic acid production.

Earlier in 1990 in the International Journal of Sports Medicine, Brouns observed in a number of subjects that exhaustion, when performing endurance exercise at high intensities, occurred when plasma ammonia levels were high (7). Muscle cramps occurred in subjects who reached their highest individual ammonia values and seemed not to be related to serum potassium, plasma lactate, or muscle glycogen. He hypothesised that high intra-muscular ammonia levels may be related to the aetiology of muscle exhaustion and muscle cramping during highly intensive endurance exercise.

As the leaders approach the summit the temperature will have dropped to around 5 or 6 degrees Celsius, hopefully fine. But the riders can easily find themselves in misty rain or even snow so it is important to don jackets or stuff newspaper up the shirt front to keep warm on the hair raising switch back descent. Hurtling down these mountains at 80 - 90kph there is not much chance to eat or drink but it is important to keep the legs ticking over in order to flush as much lactic acid from the muscles as possible. Back down in the valley, its straight across the main road and the start of the climb on the other side. The 20km climb up the Col de Glandon to 1900m. During the climbs the top riders will be generating over 400 watts of power but obviously weight plays a big part in how fast they can climb. The lightest riders are a mere 55kg, the heavier riders at 75 to 80kg. Carrying an extra 20kg up these mountains is no fun and it's easy to tell who will be at the front and who will be at the back.

Weight is not the only thing. Oxygen is vital to the metabolic process of converting sugars into energy so that the muscles can produce the power. Lung capacity and cardiac efficiency are important factors in delivering that oxygen as well as flushing out the waste products. Professional cyclists have been shown to have lung capacities much higher than most other athletes do. This allows the cyclists to suck up oxygen at between 4.5 and 6.5 L/min. Axel Merckx; son of the famous Eddie Merckx has the biggest lung capacity of the riders in this year's Tour at 8.17 litres. A study carried out on riders in the 1995 Tour confirmed earlier reports that the left ventricular mass in professional cyclists was double that of a control group and the mean arterial diameter was 13% higher (8).

Back on the bike, the leading contenders along with their strongest teammates are descending the Col du Glandon. The pace starts to increase with only 30km to go to the finish. Time to take in fluid and energy gels prior to the final climb. You can't afford to run out of energy stores at this stage. Through the little village of Bourg d'Oisans. Half a kilometre out of town, turn left and there it is. L'Alpe d'Huez. Fourteen kilometres straight up the side of a mountain to the ski resort at 1880m. The climb is famous for its 21 hairpin bends and 8.1% gradient. A spectacular place to finish a bike race.

There is one rider 6 minutes ahead. A few kilometres up the climb Armstrong attacks. His heart rate goes from 170 to over 200 beats per minute and he will be in the red zone, above Anaerobic Threshold. Over the next three kilometres he establishes a lead over Ullrich. Legs almost certainly burning with the lactic acid. Mine would be anyway. And then he settles down to a steadier pace, spinning in a small gear. Get rid of the pain. Get the heart rate under control. Half way up and Armstrong has caught the leader and he surges past "just like a motorbike". A kilometre from the finish and the crowd urge him on. Lance Armstrong races away to a superb victory. Two minutes ahead of Ullrich. The climb has taken 38 minutes at a stunning average of 21.5 kph and that's after riding 200km over two other mountains. Last year I had the good fortune, although some might question my sanity, of riding L'Alpe d'Huez. It took me 70 minutes, nearly double the time and half the speed.

After the stage there is plenty to be done to ensure the riders can do it all again the next day. Within half an hour of finishing there will be a high protein drink with possibly some Branch Chain Amino Acids to help repair muscle damage. If a rider has completely depleted the stored glycogen there is also the danger that his body will start breaking down muscle protein to provide a source of energy. Glycogen stores need to be replaced as quickly as possible as the immediate period post exercise is the time when maximum uptake occurs. Drinks high in Carbohydrate start the process of fuel and fluid replacement. In extreme cases a rider may require a drip. After a shower and massage it is time for the 14-cheeseburger dinner.

As we know, Lance Armstrong went on to win the Tour de France and what did he receive for his trouble. 7 million NZ dollars. The overall prize money for the Tour de France is 50 million NZ dollars. Second gets $3.5m, third $1.8m, stage winners a mere $160,000. The prize money is well spread out to ensure there is an exciting spectacle. Unfortunately, as we see in many sports, high stakes often lead to high risks. And professional cycling has had more than its fair share of bad publicity. Drug taking has been going on for many years. It probably started with something as innocuous as cigarette smoking or more likely caffeine. Old time cyclists have told me that it was quite common to have a special bidon reserved for the end of a race, which contained super strong coffee, just the stimulant to the sprint home.

A far more sinister stimulant hit the scene in Europe sometime in the 1950's or 60's. Known as Pot Belge it was a cocktail of amphetamines, cocaine, heroin and cortisone. In 1998 the French police uncovered the largest amateur doping network and charged them with buying, selling and importing these drugs. One user had these words to say in court. "Initially it was for racing, because you felt much better," said one of the accused. "But when you got home, you felt tired, not well in your head. And then you wanted to start it again..." Medical experts put this dependence down to the heroin and cocaine that were present in small quantities in the pot, also pointing out the extreme risk of a heart failure to young riders.

In 1998 the French police were again involved after finding large quantities of sport enhancing drugs in the Festina team car as the Tour entourage returned from Ireland. It was this affair that really opened up the eyes of the world to the extent of drug taking and especially the use of EPO, recombinant erythropoietin. EPO is the wonder drug for endurance athletes. It is naturally produced in the kidneys and stimulates the production of red blood cells. The more red cells you have, the more oxygen you can transport to the muscles, and the more endurance performance is enhanced. The downside of course is that the packed cell volume goes up to dangerously high levels.

Some years ago a number of Scandinavian Cross-Country skiers mysteriously died and there were strong suspicions at the time that EPO or blood doping was involved. Until the Sydney Olympics it was impossible to detect EPO abuse because it is naturally occurring and breakdown products disappear from urine samples 3 days after use. Of course, anybody using EPO will take it at least several weeks before competition making its use very difficult to prove. With the advent of new detection methods, cycling has imposed strict testing regimes on all competitive cyclists. To my knowledge it is the only sport conducting regular blood as well as urine testing on athletes. If a cyclist's haematocrit level is above 50% they cannot ride for three weeks and through the implication that they are taking EPO, anyone found guilty is usually found in the dole queue.

An interesting new drug appeared recently in the Giro d'Italia. A cyclist was found to have a drug called RSR-13 in his possession although it is not available outside the United States and is for research only. RSR-13 is still undergoing experimental trials for legitimate uses in cancer therapy especially brain and lung cancer as it enables more oxygen

to be released to the tissues. It achieves this by shifting the oxygen dissociation curve in a similar way to increasing 2,3 DPG levels.

Fortunately I cannot see this drug being of any practical use to a cyclist. Increasing the ability of haemoglobin to give up oxygen also decreases its ability to take up oxygen from the lungs. Patients being treated with RSR-13 require pure oxygen to ensure adequate uptake. I doubt if you will find any cyclists keen on carrying an oxygen cylinder in their back pocket. As well as that little problem its effects only last three hours and it is easily detectable in urine samples. So while it is unlikely we will see this drug being used in sport it causes great concern that these highly tuned, extremely fit young men continue to search for any advantage over their fellow competitors in the quest for the almighty dollar. Not something most medical laboratory scientists will have to worry about.

I have a short video clip of the famous "Hell of the North" bike race from Paris to Roubaix. It is raced in the Northern Hemisphere spring and the riders have to negotiate more than 20 sections of cobbles often no more than farm tracks. As is usual in Belgium at this time of the year it rains and rains and rains. Much like Auckland in spring. The combination of rain, mud, slippery cobbles, cold weather and tyre punctures results in a high attrition rate with only the strongest and fittest surviving. The cobbled sections are often narrow and as you can imagine, very uneven. The slip streaming effect of a large group of cyclists is largely negated as the riders try to negotiate the easiest route in single file often taking to the verges along the side.

To me it epitomises what this sport is really about. Hours of training, sheer determination, dedication to their profession and a fair degree of natural ability. Like these cyclists I would urge you, as medical laboratory scientists, to take up the challenge, rise above adversity and perform to your utmost in the very best professional manner, despite the conditions surrounding you.

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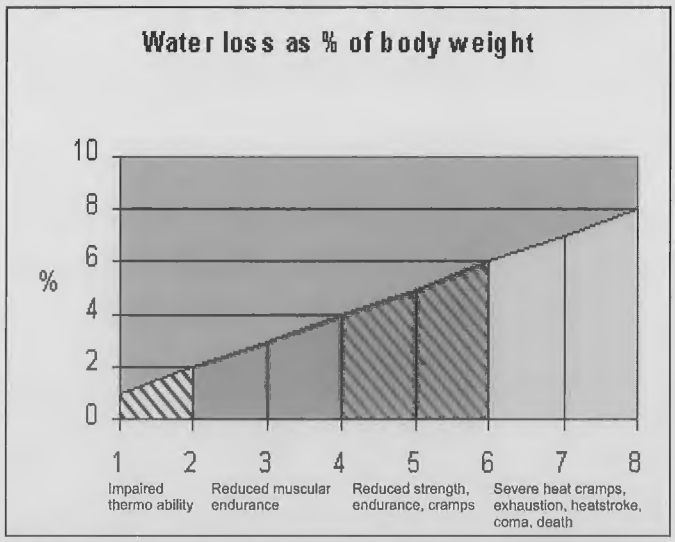

Write a scientific paper - the easy way

Tim Albert, BSc, FCIPD. Principal, Tim Albert Training, Dorking, UK

One of the most striking things about getting published in medical journals is that everyone assumes it is so hard to do. And in many cases it is. A junior member of the team will be assigned to do most of the hard work. He or she is given a mass of data and some vague instructions, and largely left alone to write. When they have finished everyone else is empowered to shower all kinds of criticism on the draft, from the misplacing of an apostrophe to the suggestion that the article be completely rewritten for another journal (with a higher impact factor, of course!). Not surprisingly, many people simply give up: one of the saddest aspects of the courses I run is the number of those who admit to one or more "bottom draw papers" - articles that have been written, but are now stalled and lie forgotten at the bottom of a desk.

The purpose of this article is to redress the balance, and to make it clear that writing a scientific paper - and getting it published - is a lot easier than people would have you believe. What you need to do is approach it systematically; I have already advocated a 10-stage process (1) and here is a summary.

Step 1: understand the publications game

Writing a scientific paper predicts little than other than the ability to write another scientific paper. It shouldn't be taken as a measure of your clinical or scientific ability, your intelligence or your worth as a human being. Those who get published are those who have the motivation (see step 2, below) and have worked out what they need to do to get published.

The key here is not to aim to write a 'good paper' (the difficulty with this is that everyone seems to have different opinions about the 'good'), but to write a paper that an Editor will want to publish. Articles have to be sound scientifically, but there are more of these than there are spaces available to be published. So the ones that are chosen are the ones that Editors think their readers will most appreciate. It follows that, if you want to be published in a particular journal, you should try to find out what the editor wants - and meet that demand.

In other words, this is essentially a sales activity. Your task is to make a product (the scientific paper), and sell it to the customer (the editor). Once he or she has bought it (publication) then you have completed the transaction and succeeded in your task. In other words, you have won the game.

Step 2: decide whether you want to play the game

The most important component of a scientific paper is the first author. So if you set out on this trail you must be sure that you can commit to the large amounts of time you will need. This in turn means that it is something you really want to do.

Ask yourself two fundamental questions. Why do you want to write a scientific paper? In which journal do you want to be published? Keep focussed on your answers because having a clear idea of what you want to do - and how - will help to keep you motivated. If you have no good answers to these questions, then forget about writing an article, and do something more useful (such as looking after patients) or more enjoyable (such as reading or walking).

Step 3: set your brief

This is a vital stage, but often overlooked. Resist the temptation to start writing up your results as soon as you have collected them. Take time to think about them: what do they really mean? Write down the message as a single sentence in about 12 words, with verb. This will force you to think clearly, and also help you to build up the article from a central core.

A message you think is interesting is not enough in itself. Will an editor think it interesting enough to publish? This should be an evidence-based decision: look for journals that have published on this topic (easily done through a literature search) and try to identify those who have had an obvious interest. In particular look for a 'conversation' where the last paper has asked the question that you are now in a position to answer.

Finally, work out who the authors will be - and agree with them, preferably in writing, not just the message and the target journal, but also the timetable. This will help considerably later on, when co-authors start to suggest that you do the work all over again in another form for another journal.

Step 4: collect your information

One of the major problems of writing is not what to put in but what to leave out. Many scientists favour the 'leaf shuffling' approach (2), in which they collect all the data and then shuffle it around on the screen until it finally takes on the shape of an article.

There is another way, which is to take a large piece of paper and write your message in the middle. Then collect around this the questions and answers you need to support this message. This is the technique known as mind-mapping (3). It will help you sort out what you will need to say, and what you can leave out.

Step 5: draft a plan

Writing is linear, so you will need a plan. First look in your target journal and see what kind of structure the existing articles follow. Look in particular for the sections. These are usually four: Introduction (why did you start?), Methods (what did you do?), Results (what did you find?) and Discussion (what does it all mean?). Work out roughly how many paragraphs there are (typically two for the Introduction, seven for the Methods, seven for the Results and six for the Discussion [1]).

Table 1. Some methodological questions

• Have you sought the opinion of a qualified methodologist/statistician?
• Have you chosen an appropriate study design?
• Have you studies enough people, rats or whatever?
• Has the work been done before?
• Do your results really support your conclusions?

Table 2. Some ethical questions

- Ethics: have you obtained ethics committee approval?
- Authorship: have you (as far as reasonably possible) ensured that all authors have had a 'significant intellectual involvement'?
- Duplicate publication: has this work been published elsewhere? If so (for instance in another language) make sure to mention this in your covering letter, and to reference it in the text
- Plagiarism: is the work your own
- Fabrication: do you have the evidence to support your claims?

Look at whether there are key sentences (for instance at the end of the Introduction and beginning of the Discussion). The first sentence is usually a 'mini-seminar' of all we know already, and is usually boring and formulaic. More important are the last sentence of the Introduction ('We therefore set out to...') and the first sentence of the Discussion ('Our study clearly shows that...'). The last sentence should be a clear exposition of your message.

Step 6: write

Many people spend far to long at this stage, mainly because they are not just writing, but planning and revising at the same time. If you have done the thinking in advance (as suggested above) then you will be able to write very quickly - an Introduction in 10 minutes, for instance.

The advantage of this is that you won't have any time to worry about the details, and you should end up with a first draft that is clear and coherent. Most important, it will be down on paper. At this stage, follow the advice of the American writer James Thurber: 'don't get it right; get it written' (4).

Step 7: rewrite

This is a vital part of the process. Unfortunately most people do it very badly, by spending far to much time on the things that matter least. So the first thing to do is to ask the big questions. Does the article have a coherent message? Is this message appropriate for the target journal? Is it structured appropriately (see step 5 above)?

Then comes the micro-editing details. Go through the manuscript a few times, doing specific tasks each time. Are the facts checked and double checked? Do the figures add up? Have you met the basic rules of grammar and spelling? Have you followed the requirements in the Instructions to Authors? Have you made any stupid errors, such as 'night' for 'might' or 'pager' for 'paper'? This is tedious work, but has to be done.

Step 8: add the extras

This is more tedious work, and you may wish to divide it into bite-size pieces.

• **References:** go through the draft and work out which statements in your text need to be supported with references. If there are several papers that will make the same point equally well, favour those published in your target journal, or even better those that have been written by the Editor of your target journal, as here (5).

• **Tables and figures:** these should provide the data needed to support the text. Look in your target journal to see what kind of tables and figures they like, and what style to follow.

• **Title:** Editors have very different views as what makes a good title. Some will like semi-colons, others will like verbs, still more will like question marks. Look through past editions and see the most prominent style. Then follow it. It should take you less than a minute.

• **Abstract:** write the abstract as late as possible - and in one go. Again follow the style of your target journal - and it shouldn't take more than 10 minutes. Make sure you are being consistent: studies have shown considerable variation between abstract and article (6).

• **Covering letter:** this is important but often overlooked. It is your chance to sell, so tell the Editor who you are (this should come from the letter head, qualifications, etc), what you are sending (the message), and point out tactfully why he or she should publish it. For instance, if your article is the last in a 'thread' or conversation that has been going on in the journal, say so.

Step 9: internal reviewers

Ask your colleagues for informal advice, but not to much. Instead of inviting them to make as many comments as they like, direct them to one specific task only. Can you see any stupid errors in here? Are there any major omissions? Would you (as reader of the target journal) understand it?

Then show it to your co-authors, again asking them wherever to do specific tasks, rather than issuing blanket invitations to criticise. Remind them of the deadlines you have agreed (step 3). Try to make any discussion evidence-based: a dispute about whether the article should be in the active or passive ('We discovered...' versus 'It was discovered...') can be settled simply by looking at which is favoured by the target journal.

Step 10: send it off

This is when you should celebrate, because from now on the matter is largely out of your control. If the science is poor and you have failed to pick a suitable journal (both of which are easily preventable, see step 3 above), your article will be rejected. If the science is really poor and can't be improved, then chuck it in the bin. If the science is considered acceptable, then you have made a marketing error: look for a more suitable journal and go back to step 3.

One day you will be told that your article is accepted - provided you take into account the comments of the reviewers. This is sometimes easier said than done, especially when they disagree or have misunderstood. Remember they advise the Editor; so do what you can. If you feel a change is wrong, then tell the Editor, and give your reasons.

With any luck the next stage will be publication. Be prepared for all kinds of critical comments. Remember, however, that it is a lot easier to comment on something that has been written than it is to write something in the first place. So treat yourself to another celebration.

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Table 3. Ten pompous words to avoid and suggested alternatives

Words to avoid	Suggested alternatives
additional	more
approximately	about
assistance	help
elevated	raised, higher
frequently	often
following	after
novel	new
participate	take part
possesses	has
sacrifice	kill

Table 4. Ten pompous phrases to avoid and suggested alternatives

Pompous phrases to avoid	Suggested alternatives
affect in a positive way	benefit
a number of	many
at this point in time	now
due to the fact that	because
in addition to	also
in the event of	if
prior to	before
male paediatric patient	boy
upper limbs	arms
subsequent to	after

Further reading

This paper is based on the book Winning the Publications Game by Tim Albert (2nd edition, Radcliffe Medical Press, 2000). For a sound view of what you should be doing before starting research, read Trishia Greenlagh's How to Read a Paper (2nd edition, BMJ Publications Group, 2001). If you want to learn how to write in a good English style, the classic work is The Elements of Style by W Strunk and EB White, now in its 4th edition (Allyn and Bacon, 2000). For some sensible advice on editing for style, try Medical Writing: a Prescription for Clarity by Neville Goodman and Martin edwards (2nd edition, Cambridge University press, 1997).

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Is the TRACE(tm) spectrophotometric method specific and precise enough to replace flame photometry for the analysis of lithium?

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Abstract

Objective Many methods are used for measuring serum Li^+, including flame photometry, atomic absorption, ISE, and lately spectrophotometric methods. In order to consolidate laboratory equipment, a method comparison for serum Li+ was conducted between a spectrophotometric method (TRACE™) and our current method, flame photometry.

Methods Serum was collected from 42 patients on Li^+ therapy and analyzed in duplicate on an automated analyzer (Hitachi 917 analyzer, Roche Diagnostics) using a spectrophotometeric method (TRACE(tm) Scientific), and by flame photometry (IL 943).

Results Regression coefficients for the data showed good correlation. The slope of the Deming regression line was 0.978 and the intercept was 0.0224. The Bland and Altman difference plot showed that there was little mean difference, systematic or proportional bias. Within-batch coefficients of variation (CV) at Li^+ values of 0.6 mmol/L and 1.2 mmol/L were 4.47% and 1.91%, and 2.2% and 1.5% for flame photometry and the spectrophotometric method respectively. Between-batch CV at Li+ values of 0.68 mmol/L and 2.05 mmol/L were 3.34% and 3.20%, and 1.4% and 1.1% for flame photometry and the spectrophotometric method respectively. The selectivity study showed negligible interference from Na^+. Cost comparison showed that the spectrophotometric method was more expensive but required less operator time.

Conclusion The TRACE(tm) spectrophotometric method shows adequate correlation and precision and would be suitable as a replacement for flame photometry for serum Li^+ analysis.

Key words: Lithium, spectrophotometry, flame photometry, comparison

Introduction

Since the discovery in 1949 of the efficiency of lithium carbonate in manic-depressive psychosis it has become the first choice in the treatment of this condition (1). It is commonly used in the treatment of acute manic-depressive episodes and can be used long term to prevent illness recurrence. Lithium is prescribed to approximately 1:130000 people in the population. Therefore the potential for Li+ toxicity needs to be closely monitored during treatment. For this, it is essential that Li^+ serum concentrations are measured accurately and rapidly by the laboratory.

In 1954 the process of monitoring serum Li^+ concentration was implemented. It was discovered that Li^+ has a half-life of 20hrs in the body and is absorbed from the gastrointestinal tract within 2-4 h when plasma concentration is at its peak. The measured concentration of Li^+ varies greatly during the day, therefore it was decided to make a standardized twelve hour post dose measurement to give comparable results from one determination to be effective for most patients (1). A level of 1.2-1.5mmol Li^+/L now signifies a warning range for medical professionals and a level above 1.5mmol Li^+/L indicates a significant risk of intoxication. It was concluded that a 12-hour plasma Li^+ of 0.5 to 0.9mmol/L is in the therapeutic range.

Thus serum Li^+ concentrations are routinely monitored to ensure both patient compliance and to avoid serious intoxication. It is because of the narrow therapeutic range for Li^+ and dangerous toxic side effects that doctors need to constantly monitor doses by observing serum Li^+ concentrations. Lithium doses are calculated by taking into consideration body weight, age, sex, and renal function. Lithium is often given as a single daily dose and is measured 12hr post dose. These measurements have historically been conducted by atomic absorption and flame emission spectrometry. However, spectrophotometric Li+ methods have recently been developed. They have the advantage of greater turn around time, greater specificity and consolidation of laboratory equipment with minimal extra staff training required.

The aim of this study was to compare flame photometry and the TRACE(tm) spectrophotometric method for the measurement of serum Li^+. Also, to determine whether the photometric method is specific and precise enough to be able to be considered for use in the laboratory. Cost, speed, and operator training were also taken into account.

Methods

Blood samples were collected over a three-week period (without anticoagulant) from 42 patients on Li^+ therapy and centrifuged at 5000rpm for six minutes. Serum was separated and stored at -20°C in plastic tubes. On the day of analysis the samples were thawed and vortexed. The samples were then analyzed for Li^+ content in duplicate by flame photometry (IL 943) and by the TRACETM spectrophotometric Li^+ method on a Hitachi 917 analyzer (Roche Diagnostics). Application parameters were loaded onto the Hitachi 917 according to the manufacturer's specifications. The assay was then calibrated and controlled to ensure it met the laboratory's defined limits of performance.

For within batch precision, two patient samples of about 0.5mmol Li^+/L and 1.0mmol Li^+/L were analyzed 15 times each by both methods. These patient values were chosen as they lie at the bottom and top of the therapeutic reference range for serum Li+ respectively. For between batch precision, controls (high: 2.05mmol Li+ /L; low: 0.6mmol Li^+/L) were run over a three week period. To assess whether Na^+ interferes in the spectrophotometric Li+ method, as has been noted previously (1), 10 saline (0.9% NaCl) samples were analyzed for Li^+.

Both instruments were calibrated with a standard solution containing 1mmol Li^+/L. The quality control material used was Liquicheck(tm) human unassayed chemistry control (Cat #691 and #692, BioRad Diagnostics). Quality control and calibrations were performed according to manufacturers specifications before each run of samples on the Il 943 and the Hitachi 917.

Agreement between methods was investigated using correlation coefficients. Linear regression and Deming regression were employed to measure the association and agreement of the two methods. Bland and Altman difference plots were used to show the actual statistical difference between the two methods (11).

Staff time involved in conducting the two methods was assessed by timing the routine maintenance, QC/calibrator and the processing itself. The cost per test for the flame method was calculated by adding up all items purchased for the IL 943 in a six month period, such as gas, reagent, controls and cleaning fluid, and then dividing the total by the number of test conducted in that time period. The cost of each spectrophotometric Li^+ determination was calculated by the cost of the reagent alone. Small amounts of control and calibration material are also used at a minimal cost.

Results

Deming regression is now the favored statistical technique for methods comparison analysis due to the fact that it allows imprecision in both methods, unlike linear regression which assumes no variation in x (3, 10-12). It takes into account the variance of the two methods by making them into a ratio and is able to give a level of specific agreement between alternative clinical methods rather than an association. Demings regression showed a strong agreement between the two methods with an equation of Y = 0.978x +0.0224. Weighted Deming regression (n=42) showed that the line of equality and the line of best fit were nearly identical; slope = 1.050 (95% CI: 0.881 - 1.218); intercept = -0.0159 (95% CI: -0.1098 - 0.0780). There were, however, a few outliers that should be investigated. Both of these regression studies show a strong relationship between the two methods.

The ineffectiveness of other methods to be able to assess the actual difference between methods has lead to the development of the Bland and Altman difference plot for method comparison studies (4). These plots visually examine the relationship by plotting the difference between the two methods against the mean of the two methods. This allows for the assessment of bias (do the differences differ systematically from 0), the error (how much do the differences vary) and to spot any relationship between the differences and concentration. If the methods are comparable, the differences are clustered equally around zero and the mean difference is low. This was the case in this study where the mean difference was 0.00833 mmol Li^+/L (95% CI: -0.0104 - 0.027). The results also showed an approximate symmetry of the difference around zero, which would indicate there is no systematic bias. The methods are also shown to have little proportional bias, as with increasing concentration, the difference does not show an increase.

The precision of the differences can be described by calculating the limits of agreement. I have defined limits of agreement as 1.96 SD limits because then 95% of the differences will fall within these limits. The "limits of agreement" are calculated by the mean difference +/- 1.96 times the standard deviation of the differences (4). In this case the mean was 0.00833 mmol Li^+/L, SD was 0.0597, making the "limits of agreement" -0.111 mmol Li^+/L to 0.125 mmol Li^+/L. This difference is very small and has no clinical significance as it would not change the interpretation of a result in the therapeutic range of 0.5 - 1.0mmol Li^+ /L.

Within batch precision was calculated on a batch of 15 low (0.6mmol Li+ /L) and 15 high (1.2mmol Li+ /L) patient samples, analyzed on both the IL 943 and the Hitachi 917. The resulting CVs for the spectrophotometric method were 1.52% and 2.25% for the high and low samples respectively. The manufacturers claim CVs of 1.25% and 2.07% for the high and low respectively (TRACE(tm) lithium insert). For the IL 943 CVs were 4.47% and 1.91% for the high and low samples respectively. The manufacturer claims CVs of 2.5% at all levels.

For between batch precision, low (0.68mmol Li+ /L) and high (2.05mmol Li^+ /L) control values were collected daily over three weeks for both methods. The resulting CVs for the IL 943 were 3.34 % and 3.20% respectively for the low and high controls. Comparative CVs for the Hitachi 917 were 1.37% and 1.06% respectively. The CVs for the Hitachi 917 showed greater levels of precision, both within and between run, compared to values achieved for the IL 943.

To assess interference by Na^+, 10 aliquots of 0.9% NaCl were analyzed for Li^+ the TRACE(tm) method. All, but one resulting value gave a negative value, suggesting no interference by Na^+. One of the saline samples returned a value of 0.01mmol Li^+ /L, which is of no clinical significance.

Maintenance for the Hitachi 917 analyzer was negligible as it is carried out in the morning for all tests conducted on the analyzer. The whole process takes approximately 20 minutes for 30 tests on the Hitachi 917, resulting in less than 1 minute per test. The IL 943 requires cleaning with diluent after every use and the gas bottle and reagents need to be changed regularly. Calibration and QC time is also negligible on the Hitachi 917 as it is carried out automatically at start up or can be programmed when needed. The IL 943 requires calibration and quality control at the start of each batch and these must be retrieved and run before a sample can be analyzed. The process itself s also very minimal on the Hitachi 917 at approximately 5 minutes compared to 10 minutes on the IL 943. Overall, the Hitachi 917 was more efficient than the IL 943, in terms of operator time.

The cost of analyzing Li^+ on the flame photometer was calculated on the basis of the equipment purchased over a six-month period. This included gas, diluent, cleaning solution, controls and calibrators. The total cost was $NZ1155.42 for 396 samples, or $NZ2.92 per sample. The spectrophotmetric method costs were calculated on the cost of the reagent alone. The cost of $NZ840 for 2 x 28mL bottles of reagent results in a cost of $NZ3.65 per sample.

Discussion

The main disadvantages of flame photometry are speed, the bench area requires, and maintenance time. For an instrument that in our laboratory is only used to analyze serum Li^+, with all of its attachments, such as gas cylinders and compressors, takes up a large amount of valuable space (60 cm of bench space with extra floor space for the compressor). Operation time is also long, it can take up to ten minutes for start up procedures and approximately another 5-10 minutes to analyze 3-5 samples. The operation and cleaning of the flame photometer is relatively technical and requires specific staff training. The noise produced by this instrument is also quite excessive for a small laboratory. The flame photometer also requires the reference solution and gas bottle to be changed frequently (about once every 2-3 weeks) requiring further staff time. Calibrators and control also have a high usage per reportable result as a set amount is required for even a small batch of samples. A drift in control values during a large run can occur and invalidate results. However, this is not usually a problem in our laboratory, due to small batch numbers. The cost of each individual Li^+ measurement was $ NZ$2.92, which takes into consideration the gas, calibrator, cleaning products and test solutions.

The main advantage of measuring Li^+ by spectrophotometry on the Hitachi 917 analyzer is consolidation of laboratory equipment. At present there is a concerted effort to get separate laboratory tests all on a minimal number of analyzers in order to make more laboratory space available and lessen operator time (5). The Hitachi 917 allows for this by removing the flame photometer and requires minimal further staff training. Other features of the Hitachi 917 Li^+ method includes low sample volumes (5 µL, compared to 20 µL on the IL 943). This is very useful when only a small sample is received, or many tests are required. The Hitachi 917 is also random access, which means that calibration and quality control can

be conducted once in the morning, thus validating the assay for continual use for the rest of the day. This is unlike the IL 943, which is a batch analyzer and requires new quality control and calibrators at the start of every run.

However, the cost of each Li^+ test on the Hitachi 917 is slightly higher at $NZ3.65 due to the high cost of the TRACE reagent. The cost of the reading unit on the 917 does not need to be taken into consideration due to the fact it is already in place for other tests . However, extra quality control material and calibrators will be used, but the cost of these are minimal. One disadvantage of the TRACE reagent is that it is light sensitive and absorbs atmospheric CO2. This requires the reagent to be stored capped in an opaque container when not in use for prolonged periods. The reagent also takes up vital space on the Hitachi 917's reagent disk for a very limited number of tests. To overcome these issues the reagent should be placed on the Hitachi 917 and removed and placed in the refrigerator when not in use. Samples for Li^+ analysis can also be run in larger batches on the Hitachi 917, unlike the IL 943, which begins to experience drift with high test numbers (15+).

In conclusion, the results of this study have shown a high degree of correlation between the TRACE spectrophotometric method and flame photometry for serum Li+. Even when small differences are taken into consideration, most are not clinically significant. The Hitachi 917 spectrophotometric Li^+ method was shown to be highly specific and precise. Comparison of the two methods showed that, although the cost of each individual test is higher on the Hitachi 917, the operational costs, including numbers of operators and time, is lower. Use of the Hitachi 917 also has the added advantage of allowing the removal of the flame photometer in our laboratory, which only carries out one test and takes up a large amount of space. The Trace™ spectrophotometric Li^+ method on the Hitachi 917 analyzer shows adequate correlation and precision and is a suitable alternative to flame photometry.

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Assessment of tumour outcome using immunohistochemical and histochemical techniques

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Abstract

During the past one hundred years, a variety of techniques have been established in an attempt to provide prognostic information from standard haematoxylin and eosin stained sections for the management of malignancies. These techniques include the development of grading systems and the quantitation of mitotic figures in malignant tissues. Both methods, while important and novel in their time, have their limitations. The advent of immunohistochemical techniques, in conjunction with histochemical methods has allowed the more accurate assessment of the proliferation rate of tumours as these methods are more specific and quantifiable. This review of my published papers explore a number of variables in order to determine the correlation between expression of various antibodies and tumour prognosis.

Proliferative rate was investigated using a silver staining method for Nucleolar Organiser regions (AgNOR), and immunohistochemical detection of Proliferating Cell Nuclear Antigen (PCNA), and the most specific cell cycle label, Ki-67 antigen. Factor Viii (von Willebrand Factor), an antibody present in endothelial cells, was used to determine whether vascularity was of prognostic significance in renal cell carcinoma. Luminal Epithelial Antigen (LEA.135) is an antibody present on luminal epithelial cells in normal breast and glandular epithelium of other organs, including colon. Our studies were conducted in order to determine if this antibody was of utility as an indicator of progression for both colonic adenocarcinoma and transitional cell carcinoma of the bladder. S-100 protein demonstrates the presence of Langerhans cells, which are pivotal in the development of a tumour-mediated immune response. This antibody was used to determine the relationship between tumour proliferation, Langerhans cell numbers, and the presence of lymph node metastasis in a series of breast carcinomas. Tumour proliferation was measured using the Ki-67 antibody in the same series of tumours.

The results of these studies showed that these markers were of variable value as prognostic indicators. Proliferation markers were shown to be of importance in determination of tumour prognosis, while other antibodies appeared to have only a limited value in determining tumour progression. This review details the findings of ten separate published studies. The materials and methods utilised and results obtained are presented, while the prognostic value of the tumour markers studied is discussed in detail.

Key words: tumour markers, AgNOR, PCNA, Ki-67 antigen, von Willebrand Factor, LEA.135, S-100 protein

Introduction

The role of pathology in the management of disease is a relatively novel concept. Rudolf Virchow, the father of pathology, was the first to recognise that different conditions showed morphological changes according to the state of the tissue (1). He struggled to convince sceptics that the histologic examination of tissue could not only be used as a diagnostic tool, but behaviour of certain diseases could be predicted by recognising definite patterns within the tissue. Gradually, as consistent results became internationally known, this type of examination became accepted, and by the turn of the twentieth century, histopathology was an established science.

The first major step in predicting the outcome of malignancy was made by Broders who noted that while some tumours appeared to be similar in appearance to the tissues of origin, others were not (1). The first grading system was developed by Broders using squamous carcinoma of the lip. He based the grade on the proportion of well-differentiated tumour cells to poorly differentiated cells. He proposed that poorly differentiated tumours proliferated more rapidly than the well-differentiated counterparts and therefore had a less favourable prognosis.

The work of Broders initiated the development of various grading systems for many types of tumours. Although these grading systems were able to assist in prognostic assessment of some tumours, there have been many reported inconsistencies and in particular many instances are recognised in which reproducibility is a major problem (17,30,31). Most tumours fall into the middle grading categories in many grading systems, where behaviour is least predictable; thus the practicality of using grading systems as prognostic indicators is diminished. As a result of these inconsistencies proliferation markers were investigated as a tool to determine tumour outcome.

The assessment of mitotic rate was the first step in assessing tumour proliferation. This has the advantage over grading in that it uses cell proliferation as a variable that is not reliant on arbitrary endpoints. The measurement of mitotic rate has been shown to have significant correlation with survival for a variety of tumour types (2); however, the quantitation of mitotic figures has its own limitations. The most important of these is that mitosis occurs in the cell over a very short period of time and may only be seen occasionally in tumours that proliferate slowly. Other factors that influence the determination of the mitotic rate are variables associated with the expression of mitotic figures per high power field. These variables include the size of the high power field area, the degree of cellularity within that field, and the speed at which the tissue is fixed. There have been many mathematical models and counting procedures proposed to reduce the variability and increase the consistency of mitotic rate counting(32), however these have not found universal acceptance.

The advent of immunohistochemistry and particularly the development of techniques to expose previously masked antigens has been an invaluable aid in assessing prognostic outcome in tumours. Various antibodies have been used, both alone and in conjunction with other histochemical methods, to assess proliferation rate, vascularity, Langerhans cell density and the presence of epithelial and stromal antigens, all of which have been shown to have prognostic significance (1,4-6,9,10,12-14,16,19).

Fixation

Fixation is the first and arguably the most important step in the preparation of tissue for histological examination. It is essential that the tissue be preserved so that the cellular constituents are maintained in as life-like manner as possible, that putrefaction and autolysis is prevented, and that the clear staining of sections is facilitated.

In the latter half of the nineteenth century and parallel with the developments that were occurring associated with the sectioning of tissues and the science of staining, many fixatives and their properties were studied (20). It was concluded, and this conclusion still remains valid, that there is not one ideal fixative. However, it is not practical to use many different fixatives in anticipation of diverse staining methods and it has therefore, become commonplace to routinely use one fixative. Neutral buffered 10% formal saline has become the "universal fixative" (21) as morphological preservation is mostly satisfactory, the fixative is easy to use and is compatible with most histochemical stains.

Neutral buffered formal saline, however, is not an ideal fixative for immunohistochemical reactions as the fixative forms cross-links between protein end groups altering the primary and tertiary structure of antigens(22). Antibodies have been developed that circumvent these problems and are reactive in formalin-fixed, paraffin embedded tissue, although their number is relatively few and this has restricted the utility of investigations relying on statistical analysis of results obtained from fresh tissue, when in general the number of cases is limited.

The development of antigen retrieval methods using metal solutions in microwave ovens in the early 1990s was an enormous advance in the field of immunohistochemistry as high temperatures were thought to alter the cross-linking proteins, thus unmasking the antigen (7). With the use of these techniques it was now possible to detect a broader spectrum of antibodies in tissues fixed in neutral buffered formal saline.

Unmasking Solutions

In their original work, Shi, Key and Kalra, used microwave oven heated metal solutions to demonstrate the expression of a large number of antigens that had previously not been seen in formalin-fixed, paraffin-embedded tissue (7). However, other studies have shown that the reactivity of different antigens varied with the retrieval solutions used which have included urea, EDTA, citrate buffer and a range of commercial solutions (8,22).

Our studies have employed many antigens that require microwave oven heat retrieval and investigation to determine the optimal retrieval solution was undertaken in our laboratory (3). The study included the use of heavy metal solutions and 10mM citrate buffer pH 6 and, although the heavy metal solutions showed slightly superior results, we concluded that their use posed a possible health and safety issue with wide implications for laboratory staff in contact with them on a day-to-day basis. After assessing these factors, it was decided that 10mM citrate buffer was the retrieval solution of choice.

Silver-staining nucleolar organizer regions (AgNOR)

Nucleolar organizer regions are segments of genomic DNA, which encode for ribosomal RNA (5). These regions can be visualised during the interphase component of the cell cycle, and because of their association with argyrophilic proteins, structures identified by a silver staining process are known as AgNOR(33).

The development of this silver-colloid staining method has led to considerable investigation into the possible applications of the technique; however, there has been disagreement regarding the reproducibility of AgNOR staining including interpretation of results(34). Problems have occurred due to the lack of an agreed definition as to what constitutes an AgNOR for counting purposes, and additionally poor staining has made it difficult to quantify smaller AgNOR clusters (1). The staining problems have been overcome with the introduction of a gold-toning step in the staining procedure, which sharpens the staining and reduces background silver deposition (23). Definition problems have been overcome by the determination that all argyrophilic foci in a nucleolus should be counted to determine an AgNOR score (28).

AgNOR counts have proven a useful tool in distinguishing some malignant tumours from benign or reactive conditions, assisting in tumour grading and determining prognosis in individuals with malignant tumours (5).

Antibody Assessment of tumour behaviour
Proliferating Cell Nuclear Antigen (PCNA)

PCNA is an intra-nuclear polypeptide that appears to be present in maximum quantities during the S-phase of the cell cycle. It reacts with proliferating cells in a wide range of normal tissues, although its utility in tumours has been debated (1). It has also been used to determine proliferation activity in several experimental animal models (4,35).

Proliferating Cell Nuclear Antigen was first used in our studies to determine the proliferation kinetics of renal tumours in mice. The mouse model was developed using renal tumours induced with intravenous injections of streptozotocin. (4,35).

Our second study used AgNOR scores and PCNA counts to compare proliferation expression in reactive mesothelial hyperplasia and malignant mesothelioma (5) in an attempt to determine if we were able to distinguish between these histologically similar conditions that have diverse clinical outcomes.

Thirdly, PCNA was used to measure the proliferation of renal cell carcinoma in conjunction with the Ki-67 antibody and AgNOR scores (6). These results were then correlated with survival rates in an attempt to predict tumour outcome in individual cases.

Ki-67 antigen

The development of a polyclonal Ki-67 antigen has proved invaluable in the assessment of proliferation kinetics for both benign and malignant conditions. While PCNA is synthesized during the S-phase, the relatively long half-life of the antigen means that in rapidly proliferating tumours expression extends into resting (non-proliferating cells) within the Go phase of the cell cycle. The Ki-67 antigen is found in cells in all phases of the cell cycle except the G0 phase, and as a consequence has largely replaced PCNA as a proliferation marker (6). While in unfixed tissue monoclonal Ki-67 antibodies bind to Ki-67 antigens, heat retrieval using 10mmol citrate buffer is necessary for the accurate demonstration of Ki-67 in fixed tissue using polyclonal Ki-67 antibodies.

In our first study, Ki-67 antigen was used to determine the proliferation of renal cell carcinoma and the results were compared with PCNA and AgNOR staining, histologic grade and tumour stage (6). In our second study, Ki-67 was investigated as a marker of tumour progression in transitional cell carcinoma of the bladder (9), while in an additional study Ki-67 antigen was used on tissue sections of medullary carcinoma of the breast in an attempt to determine the relationship between tumour proliferation, Langerhan cell numbers and the presence of lymph node metastases (10).

Factor VIII (von Willebrand Factor)

Angiogenesis is necessary for tumour growth and the development of metastases that results from the colonisation of malignant cells. Small tumours do not require an intrinsic blood supply, however larger tumours require a vasculature that is generated from host tissues. Vascularization of the tumour host tissue leads to a phase of rapid tumour proliferation, which in turn is a pre-requisite to the development of invasive growth.

Our study employs Factor VIII expression to measure mean microvascular density and tumour microvessel area, and using these parameters we investigated the vascularity in renal cell carcinomas and correlated those results with tumour stage and grade (12).

Luminal Epithelial Antigen.135 (LEA.135)

Luminal epithelial antigen.135 is an antigen which, in normal breast tissue, is expressed on the apical plasma membrane of ductal epithelium (13,19). This pattern of staining has been found in other organs, but its importance as a prognostic marker had only been investigated in breast carcinoma. We investigated the expression of the antigen in adenocarcinoma of the colon (13) and transitional cell carcinoma of the bladder (14), and LEA.135 staining was correlated with tumour grade, stage and proliferation indices in the bladder malignancies; and with tumour grade and patient survival in colonic carcinoma.

CD-3 Antibody

CD-3 antibody is a lymphocytic antibody that reacts with the intracytoplasmic portion of the CD-3 antigen expressed by T cells and has been shown to be a highly specific marker for these cells. The appearance of this antigen may signify an early sign of commitment to the T-cell lineage and may therefore be used to indicate reactive lymphocytes (29).

This study was undertaken to evaluate T-lymphocytes as mediators of tumour growth for medullary carcinoma of the breast, and to determine any correlation between tumour proliferation, as determined by Ki-67 antigen expression, and the spread of tumour to adjacent lymph nodes (10).

S-100 Protein

The antibody to S-100 protein identifies Langerhans cells which are Type II dendritic cells. These Type II dendritic cells act as outposts of the immune system in tissues by acquiring antigens and in turn respond to a variety of stimuli. These dendritic cells carry antigens to the lymph nodes where they initiate T-cell responses. In secondary immune reactivity, dendritic cells appear to have a role in activating memory T-cells in situ (10,36).

Our study investigated the prognostic significance of dendritic cells in medullary carcinoma of the breast as markers of an immune response to the tumour.

Materials and Methods

All the studies in our series utilised sections derived from archival formalin-fixed paraffin embedded tissue blocks.

As previously discussed an extensive study was undertaken to determine the optimal solution for heat retrieval of antigens (3). 10mmol citrate buffer was the solution of choice for PCNA and Ki-67 antibodies. Pre-treatment of choice for Factor VIII, CD-3 and S100 was immersion in 0.1% trypsin. No pre-treatment was required for LEA.135 staining.

Following epitope retrieval, all methods irrespective of antibody, followed the streptavidin-biotin method (15). The first step in the method is to block endogenous peroxidase by incubation in methanolic hydrogen peroxide. This step is particularly important when dealing with tissues that have a high endogenous peroxidase content, such as tissues heavily contaminated by blood. There is some speculation that methanol peroxide treatment may be deleterious to intracellular antigens (21), but this did not prove to be the case for our studies.

Non-specific binding was blocked using Dako Protein Block Serum, which is used for tissue that is susceptible to high background antibody staining. The block is casein based which has been shown to decrease non-specific binding of primary antibody and secondary reagents in immunohistochemistry.

Incubation with the primary antibody followed. Before each antibody was employed on the test sections, graduating dilutions of antibody were tested on control tissue to determine the optimal dilution factor for that antibody. With the exception of Factor VIII and S-100 protein, the antibody was incubated overnight thus allowing for the use of a lesser concentration of antibody.

After incubation with the primary antibody the sections were well rinsed and biotinylated secondary antibody added. This antibody was either swine-anti-rabbit, or rabbit-anti-mouse depending on whether the primary antibody was raised in rabbit or mouse respectively. Formation of the avidin-biotin complex followed where the biotin attached to the secondary antibody forming an insoluble complex. After washing, the reaction was visualised with 3,3'diaminobenzidine resulting in an insoluble brown precipitate. Haematoxylin was used as a counterstain.

Normal lymph node was used as a positive control for PCNA, Ki-67 and CD-3; normal breast tissue was used for LEA.135; and no specific positive section was necessary for Factor VIII and S-100 protein as the normal tissue in each section was assessed for staining of these antibodies. Tris buffered saline was substituted for primary antibody as a negative control for all antibodies.

The use of biotinylated rabbit-anti-mouse secondary antibody with mouse tissue produces some background staining due to crosslinking and for this reason, IgG 2a was used to minimise cross-reactivity.

Positive and negative controls were employed for every immunohistochemical run to ensure quality control.

Results and discussion

AgNOR scores were derived by counting discrete argyrophilic foci within each nucleus. A mean AgNOR score was derived following evaluation of 100 nuclei.

Proliferating Cell Nuclear Antigen

We primarily used PCNA together with AgNOR staining to determine the proliferation kinetics of renal tumours in mice. Both PCNA expression and AgNOR staining had previously been used to evaluate proliferative activity in a variety of pre-malignant and malignant lesions (4); however there appears to be interspecies variation in the distribution of AgNOR sites. There appears to be no such variation in PCNA expression(37).

PCNA is expressed in a relatively small compartment of the cell cycle and in slowly proliferating tissues PCNA positive cells may be encountered only rarely. If small numbers of cells are examined, it may be that some sections are not representative of cells expressing PCNA. In these cases the proliferative activity may be under-estimated (4).

PCNA indices were derived by examining 1000 cells, with the index being the percentage of positively staining nuclei.

When evaluating the mean PCNA indices and AgNOR scores, a stepwise progression between normal tubules, dysplastic tubules, papillary tumours and solid tumours was shown (4). The mean PCNA indices were 0.99, 1.65, 3.89 and 6.80 respectively, while the AgNOR scores were 2.44, 4.15, 5.90 and 6.94. These findings indicate a progressive increase in cell proliferation in these lesions. AgNOR staining showed significant variation when comparing dysplastic tubules with normal. This variation was not noted with PCNA staining.

This study indicated that for the two tumour types, papillary and solid renal cell carcinoma, there was a variation in proliferative activity in that papillary tumour indices were lower than in that of the solid type of tumours. In this series it was apparent that the solid tumours displayed more malignant characteristics than the papillary type. The solid tumours were consistently larger than the papillary, developed over a longer period of time, showed more pronounced tumour necrosis and a greater degree of nuclear pleomorphism. Some tumours displayed a dual architecture with both solid and papillary elements. These findings suggest that the papillary tumours are precursors of the solid type with the ability to develop a more solid architecture.

This relationship between the two tumour types may not be fully applicable to human tumours, but it raises the possibility of similar morphological transformation in human renal papillary adenomas which are usually considered to be benign (4).

PCNA expression was also compared with AgNOR staining in reactive mesothelial hyperplasia and malignant mesothelioma. AgNOR staining showed a significantly higher mean AgNOR count in malignant compared to reactive mesothelial processes (5). However, there were technical and interpretative variations in AgNOR counting hence the interest in PCNA labelling, where evaluation of staining is quicker and less subjective. The reactive mesothelial hyperplasia cases showed a mean PCNA index of 9.67%, while that for malignant mesotheliomas was 26.9%. The difference between the two groups proved to be statistically significant ($Z=2.85$, $p=0.004$). This suggests that a high PCNA index may assist in the confident diagnosis of malignant mesothelioma in individual cases.

Ki-67 Antigen

Our first study using the Ki-67 antibody was to compare staining in a series of 222 cases of renal cell carcinoma with other markers of cell proliferation. The study showed that tumours displaying Ki-67 indices of 6% or less, and greater than 6% showed a significant difference in survival between groups for all cases. It also showed that for each Robson stage, Ki-67 and PCNA indices, AgNOR scores and tumour dissemination retained a significant association with survival on multivariate analysis (6).

In tumours where there was no evidence of vascular infiltration, nodal invasion, or metastatic spread, a low Ki-67 index was associated with a 5-year survival rate of greater than 90%. In the same group of patients, the 5-year survivals for Stage 1 and 2 tumours were 44.4% and 25% respectively, for those tumours showing higher Ki-67 indices (6).

PCNA expression and AgNOR staining showed a weak correlation with Ki-67 scores. This inconsistency was explained by the fact that each antigen is expressed in different phases of the cell cycle. This study emphasised the importance that immunohistochemical evaluation of tumour cell proliferation can make in the prediction of clinical outcome for renal cell carcinoma, with assessment lacking the subjectivity associated with tumour grading while providing superior survival-related data.

With these findings in mind, Ki-67 was used to study the proliferation kinetics of transitional cell carcinoma (TCC) of the bladder. One hundred and twenty cases of TCC were stained and evaluated according to tumour grade, including seven cases of TCC carcinoma-in-situ. There was a significant association between tumour stage and Ki-67 staining, with Ki-67 indices increasing with increasing depth of invasion (9). Contrary to the findings of other authors (16), this study found a lower Ki-67 score for carcinoma-in-situ compared to the Ki-67 score of superficially invasive tumours. This may indicate considerable variation in cell proliferation for foci of carcinoma-in-situ.

The study demonstrated the presence of a significant relationship between tumour grade and stage, and between these parameters and Ki-67 proliferation index (9). This emphasises the potential value of Ki-67 as a marker for tumour progression for small biopsies of TCC of the bladder, with both high grade and advanced stage lesions exhibiting increased levels of Ki-67 labelling.

Factor VIII

This study was undertaken to determine the association between tumour vascularity and patient survival for clear cell renal cell carcinoma, as these tumours often metastasise via the vascular route. After staining with Factor VIII, the 150 cases of tumour were assessed by two methods. The mean microvascular density (MMD) was derived, as well as the tumour microvessel area (TMA).

Each vessel identified within an aggregate of 10 consecutive high power fields (HPF, x 400) was counted to calculate the MMD. A 25-point eyepiece was used to assess the vascular area; the number of vessels falling on each of the 25 points in each HPF was recorded. Expression of the TMA was calculated by examining 10 HPF and expressed as a percentage the intersecting points crossing vessels (10 HPFs x 25 grid intersections = 250 observation points). The tumours were graded according to the degree of nuclear pleomorphism (17) and staged according to the system of Robson et al (18).

There was a significant correlation between MMD and patient survival over a 5-year follow up period (12); however, the correlation was negative, in that those tumours with higher vascular density were associated with a greater 5-year survival rate. The 5 year survival rate for tumours with (40 vessels/HPF was 39%, while that for tumours with > 40 vessels/HPF was 40%. There was a similar correlation when MMD was compared with tumour grade. TMA had no prognostic significance, nor correlation with tumour stage or grade (12). This study showed that vascular remodelling is an important component in the evolution to a more malignant phenotype for clear cell renal carcinoma, and that the assessment of tumour microvessel density provides significant prognostic information and appears to identify those tumours with established metastatic potential.

Luminal Epithelial Antigen.135

The first study using LEA.135 was to determine the relationship between LEA.135 labelling and tumour stage and grade, in TCC of the bladder. Previously the importance of LEA.135 as a prognostic marker had only been investigated in breast carcinoma (19). The expression of this antigen was found to correlate with survival over a 5-year or longer follow-up period.

We stained 96 cases of transitional cell carcinoma and 10 cases of transitional cell carcinoma in situ. The tumours were graded according to World Health Organisation criteria (25) and staged using the UICC TNM classification (26).

Our study showed that LEA.135 is expressed in normal transitional epithelium of the bladder with expression confined to the superficial luminal cell layer. Low grade tumours showed variable antigen expression with some tumours showing diffuse staining in all cells, while others showed no staining. Higher grade tumours showed loss of LEA.135 staining. There was significant correlation between LEA.135 expression and tumour stage, with tumour infiltration into the lamina propria and beyond frequently being associated with progressive loss of antigen expression (14). The mean rank of Stage 3a and 3b tumours was 26.2 and 24.8 respectively, while tumours with Stage 1 classification showed a mean rank of 50.7. These results were consistent with those reported for breast carcinoma (19).

The second study employing LEA.135 was to determine the relationship between LEA.135 labelling with tumour grade, stage and survival in adenocarcinoma of the colon. Colorectal cancer remains a major health problem in developed countries and despite the use of aggressive surgery and adjuvant therapy, patient survival has improved little in recent years.

We stained 134 cases of adenocarcinoma of the colon using LEA.135. All cases were graded and staged according to Duke's criteria (38,39).

LEA.135 staining was confined to the luminal surface of crypt and superficial epithelium in normal colonic mucosa. Tumour areas showed cytoplasmic staining interspersed with foci showing absence of LEA.135 expression. Both cytoplasmic and luminal staining of tumour

cells was observed. There was no difference in the distribution of cytoplasmic or luminal staining between high or low grade tumours. Similarly there was no significant difference in the 5-year survival of those patients. Our study showed that, while LEA.135 expression is altered in colonic adenocarcinoma, the marker has no utility in outcome prediction for patients with this form of malignancy.

CD-3

Our series of 28 cases of medullary carcinoma of the breast were subjected to staining with CD-3 antigen. A previous study by Pedersen and colleagues (27) proposed that the presence of a diffuse cell infiltrate is a diagnostic criteria for typical medullary carcinoma although their findings were shown to vary when interpreted by different observers. Our study did not confirm Pedersen's observations. We noted that the CD-3 positive infiltrate was greater in cases of atypical medullary carcinoma than that seen in typical medullary carcinoma. The variation in these findings suggests that the degree of T-lymphocyte infiltrate is not a diagnostic feature of either type of medullary carcinoma.

S-100 Protein

Medullary carcinoma comprises approximately 1% of breast tumours. It has a more favourable prognosis than infiltrating duct carcinoma, the most common tumour of the breast (10, 40-43) and is characterised by a diffuse inflammatory cell infiltrate (10,41). It has been speculated that the density of the inflammatory cell infiltrate may be connected with tumour outcome as autoimmunity may delay tumour progression (27).

The purpose of this study was to assess the relationship between tumour proliferation and Langerhans cell numbers in medullary carcinoma of the breast. Previous studies have shown that the degree of dendritic cell infiltration into tumours and adjacent tissues to be of prognostic significance. The presence of a dense infiltrate of Langerhans cells has been associated with increased survival in patients with malignancy (10,43).

We examined 28 cases of medullary carcinoma. These comprised 19 cases of typical medullary carcinoma and 9 cases of atypical medullary carcinoma. The presence of Langerhans cells showed considerable variation with their density ranging from 0 to 101/50HPF, with a mean index of 11.4. No correlation between Langerhan cell density and lymph node status being noted (10). This provides evidence that the development of metastases in medullary carcinoma is not altered by host immune reaction and therefore shows that Langerhans cell density cannot be used as a prognostic marker for medullary carcinoma.

Conclusions

Our studies have demonstrated the significant relationship between proliferation markers, particularly Ki-67 antigen, and tumour stage and grade, for renal and bladder malignancy. We established for renal cell carcinomas that a low Ki-67 index predicted a 5-year survival rate of over 90% as long as there was no metastatic spread. The assessment of transitional cell carcinoma of the bladder showed similar results and that there was significant correlation between Ki-67 indices and tumour staging as Ki-67 indices increased with the increase in tumour depth of invasion. We also showed that proliferative indices were not of utility in the prognostic assessment of medullary carcinoma.

In association with the measurement of proliferation markers, tumour vascularity was investigated to determine its relationship with proliferation, stage and grade. We found that there was correlation between mean microvascular density and patient survival in clear cell renal carcinoma.

LEA.135 had previously only been studied in breast carcinomas, and found to have importance as a prognostic marker. We investigated its relationship with tumour stage and grade in transitional cell carcinoma of the bladder and adenocarcinoma of the colon. LEA.135 loss of expression and tumour stage for TCC of the bladder was found to have significant correlation, which is consistent with the findings for breast carcinomas. Our study involving LEA.135 and adenocarcinoma of the colon showed no significant correlation with respect to staging and grading.

The presence of Langerhans cells and of T-lymphocyte infiltrate were noted in variable proportions within all tumours of the medullary breast carcinoma series. Our series indicates that neither Langerhans cell density nor the degree of T-lymphocyte infiltrate can be used as a prognostic indicator.

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Appendix

This synopsis, submitted for Fellowship of the New Zealand Institute of Medical Laboratory Science, is a result of the following publications:

1. Thornton A, Delahunt B, Holloway L. Proliferating cell nuclear antigen (PCNA) expression in archival tissue. Evaluation of microwave pretreatment. *N Z J Med Lab Sci* 1994; 48: 103-5.
2. Delahunt B, Cartwright PR, Thornton A, Dady PJ. Proliferation kinetics of streptozotocin-induced renal tumours in mice. *Virchows Arch* 1995; 425: 577-82.
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Evaluation of two rapid tests for the detection of methicillin resistance in *Staphylococcus aureus*

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Abstract

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

Methods A total of 158 tube-coagulase positive *Staphylococcus aureus* isolates was tested; 54 were methicillin susceptible and 104 methicillin resistant. Methicillin resistance was determined by oxacillin minimum inhibitory concentration (MIC), using AB BIODISK E-test strips and the presence of the mec gene by Polymerase Chain Reaction. An MRSA was defined as an oxacillin MIC >8 mg/l or the presence of the mecA gene. The two rapid tests were performed according to the manufacturer's instructions.

Results All methicillin-susceptible strains tested negative by the MRSA-Screen test and the Velogene™ test (100% specificity). Of the 104 MRSA strains, 96 (92%) tested positive with the MRSA-Screen and 102 (98%) with the VelogeneTM. The false-negative results occurred in strains with an oxacillin MIC of 0.5-2.0 mg/l.

Conclusions The Velogene™ and the MRSA Screen are both accurate, rapid and practicable assays for use in a standard microbiology laboratory. Negative results obtained with these assays, on *Staphylococcus aureus* isolates with oxacillin MIC's of between and including 0.5-2.0 mg/l should be confirmed by PCR detection of the mecA gene. The use of these rapid assays may potentially allow clinicians to reduce mortality and morbidity, shorten hospital stays, reduce financial and personal costs, reduce the unnecessary use of Vancomycin, and delay emerging antibiotic resistance.

Key words: *MecA* gene, penicillin binding protein, methicillin resistance, *Staphylococcus aureus*, latex agglutination

Introduction

The first outbreaks of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) occurred in European hospitals in the early 1960's, following the introduction of methicillin into clinical practice (1). MRSA emerged in the 1980's as a major clinical and epidemiological problem in hospitals (2). In New Zealand, (community and hospital) MRSA now comprises 1.9% of all clinical *Staphylococcus aureus* (*St. aureus*) isolates (3). Resistance to methicillin has important implications, for patient isolation, patient management, the use of vancomycin or other antibiotics and health-care staff work restriction.

Current phenotypic tests, such as disc-diffusion and agar screen method for the identification of MRSA, simple and low cost tests, are inherently inaccurate. In contrast, immunologic and molecular tests are rapid and accurate when performed correctly. The implementation of rapid immunologic and molecular tests in the routine microbiology laboratory has already had a major impact on the clinical management of infectious diseases. It is also expected to reduce health care costs and spread of antibiotic resistance (4).

The aim of this study was to evaluate the accuracy and practicability of two rapid MRSA identification methods, recently made available in New Zealand, in a routine medical microbiology laboratory. The use of these assays should enable clinicians to administer appropriate treatment to patients in a time frame that will reduce mortality and morbidity. It should also lead to a shorter length of stay in hospital and limit both financial and personal costs. Other studies of these assays will also be reviewed.

mecA

Approximately 30-50 kb of additional chromosomal DNA, *mec*, not found in susceptible strains of staphylococci is present in methicillin resistant strains. *Mec* contains *mecA*, the structural gene for penicillin binding protein (PBP) 2a. PBP2a is an inducible 76 kDa PBP that determines methicillin resistance (1). Both PBP2a and its encoding gene, *mecA*, are targets for rapid detection tests.

The two other mechanisms of resistance to methicillin in *St. aureus* are over expression of beta-lactamase and production of other modified PBPs. Over expression of beta-lactamase is probably of no clinical significance. However, strains that contain other modified PBPs, with a reduced affinity for oxacillin, may be misclassified as oxacillin susceptible by a *mecA* gene test because the strains are oxacillin resistant in-vivo, but, do not contain the *mecA* gene (5); such strains are rare and have not been detected in New Zealand to date.

Although MRSA exhibit high-level cross-resistance to all beta-lactams, strains may be homogeneously or heterogeneously resistant. In the latter case, whereas the majority of cells express low-level resistance, subpopulations of highly resistant cells may be present in small numbers (6), a characteristic that is chromosomally mediated (7).

Most clinical isolates exhibit this heterogeneous pattern of resistance under routine growth conditions. Heterogeneous strains can however appear homogeneous under certain growth conditions (1). The phenotypic expression of *mecA* is affected by a number of factors, including pH, temperature, osmolarity, upstream regulatory sequences and unlinked chromosomal genes (6).

Materials and methods

Bacterial isolates

In total, 158 isolates of St. aureus were tested: 135 were obtained from hospital and community patient's clinical specimens submitted to the Microbiology Laboratory, Medlab Central, Palmerston North between November 1999 and September 2000. Twenty-three came from clinically referred Institute of Environmental Science and Research Ltd., Porirua (ESR) isolates. The 158 isolates comprised a mixture of methicillin-sensitive St. aureus (MSSA), borderline-resistant St. aureus (BORSA) and MRSA. All isolates were identified as St. aureus by the tube coagulase test. Although the determination of Methicillin resistance in Coagulase negative strains would be useful they were not used in this study. The breakdown of the *St. aureus* isolates tested is as follows:

MSSA	Oxacillin MIC <1mg/l	10
	Oxacillin MIC 1- <4 mg/l and mec gene absent	31
BORSA	Oxacillin MIC 4 - 8 mg/l and mecA gene absent	13
MRSA	Oxacillin MIC 0.5 mg/l and mecA gene present	1
	Oxacillin MIC 1 - 8 mg/l and mecA gene present	19
	Oxacillin MIC 8 mg/l	84
Total		158

Susceptibility testing

Oxacillin minimum inhibitory concentration by E-test

The oxacillin minimum inhibitory concentration (MIC) was determined using the AB BIODISK E-test following the manufacture's instructions. A 0.5 McFarlane Standard suspension of the organism was inoculated onto a 2% NaCl 4mm deep Mueller Hinton agar plate (Oxoid) and incubated at 35oC for 24 hours.

MecA gene testing by Polymerase Chain Reaction

The presence of the *mecA* gene was tested by ESR using a Multiplex Polymerase Chain Reaction (PCR) assay (8) on isolates with an oxacillin MIC of between and including 1.0 and 8.0 mg/l. Because other investigations have shown that an MIC of <1 mg/l is highly predictive of MSSA and >8 mg/l is highly predictive of MRSA, PCR testing for the presence of mecA gene was not performed on these organisms (9). This PCR result was used as the 'gold standard' against which the rapid tests were compared.

MRSA-Screen test, (Denka Seiken Co., Ltd., Tokyo, Japan.)

The MRSA-Screen test is a slide agglutination assay using latex particles sensitised with a monoclonal antibody against PBP2a extracted from test isolates, giving an agglutination reaction visible to the unaided eye. The test was performed according to the manufacturer's instructions:

Sample preparation:	Innoculate the test organism onto a Columbia agar supplemented with 5% Horse blood (Oxoid) and incubate 18-24 hr at 35OC
Extraction:	Suspend a loopful of organism in 4 drops of extraction enzyme Boil for 3 minutes Cool Add 1 drop of extraction reagent 2 Centrifuge at 1500g for 5 min
Detection:	Mix 0.05 ml of the supernatant with 1 drop of sensitised latex and also with 1 drop of the control latex Rotate for 3 min and observe for agglutination.

All testing was performed blind to the susceptibility and mecA gene results.

Velogene™ rapid MRSA identification assay (Alexon-Trend, Ramsey, Minnesota, USA).

The VelogeneTM assay utilises a fluorescein-labeled, biotinylated DNA-RNA-DNA chimeric probe providing an Rnase H sensitive cleavable linkage when bound to the complementary sequence of the *mecA* gene. Rnase H cleaves the RNA portion of the chimeric probe when hybridised to the target DNA. The uncleaved probe (*mecA* gene negative) is detected by binding of the fluoresceinated probe to a solid surface and attachment of an anti-fluorescein antibody conjugated with horseradish peroxidase, which converts a substrate to a coloured end product.

Cleavage of the probe (mecA gene positive) prevents binding of the probe-anti-fluorescein antibody-enzyme complex, thus preventing formation of the coloured end product. A methicillin-resistant isolate (*mecA* gene present) will produce a colourless result. A methicillin-sensitive isolate will produce a blue colour. Results are produced in 90 minutes and are read visually or using a spectrophotometer.

The test was performed according to the manufacturer's instructions:

Sample preparation:	Innoculate the test organism onto a Columbia agar supplemented with 5% Horse blood (Oxoid) and incubate 18-24 hr at 35OC. Suspend colonies in Lysis Reagent Incubate at 54OC for 20 min Denature at 96OC for 5 min
Cycling:	Transfer samples to 54OC Add cycling reagent Incubate 54OC for 35 min
Detection:	Add Cycle Stop Reagent Transfer sample to microwell plate Incubate at room temperature for 10 min Wash twice Add Detection Substrate Reagent Incubate at room temperature for 5 min Add Detection Stop Reagent After 3 min read results (visually or OD 655nm)

All tests in this study were read visually and all testing was performed blind to the susceptibility and mecA results.

Other testing

Twenty isolates were chosen at random for retesting to assess the reproducibility of results on these kits. All discrepant results were retested.

Results

All the 54 MSSA and BORSA strains tested negative by the MRSA-Screen and the Velogene™ test. Of the 104 MRSA strains 96 tested positive by the MRSA-Screen and 102 by the Velogene™ test as summarized in Table 1.

Table 1. Results of rapid tests

		MRSA	MSSA and BORSA	Total
MRSA-Screen	Positive	96	0	96
	Negative	8	54	62
Velogene™	Positive	102	0	102
	Negative	2	54	56
Total		104	54	158

The discrepant results occurred on mecA gene positive isolates with oxacillin MICs of 0.5 mg/l (1 isolate) and 2 mg/l (7 isolates). The 0.5 mg/l oxacillin MIC isolate had a weak positive mecA gene PCR, Velogene™ positive and MRSA-Screen negative reactions. All retesting of discrepancies gave the same results.

The Conditional Probabilities (10) of each assay in Table 2 were calculated from the results recorded in Table 2

.

Table 2. Conditional Probabilities of each rapid assay.

	MRSA-Screen	Velogene™
Sensitivity	92%	98%
Specificity	100%	100%
False positive rate	0%	0%
False negative rate	7.7%	1.9%

20 isolates were retested to assess the reproducibility of these assays. Both assays demonstrated 100% reproducibility with the 20 isolates tested.

Discussion

These results show that the detection of PBP 2a by the MRSA-Screen Test and detection of *mecA* gene by the VelogeneTM test are highly accurate methods for the identification of MRSA. There were no false positive results given by either method. The sensitivity of the MRSA-Screen test (92%) was not as high as the Velogene™ (98%).

MRSA-screen

Of the 104 mecA gene-positive isolates, 8 tested negative by the MRSA-Screen. All the false negative results occurred on MRSA isolates with an Oxacillin MIC of <4 mg/l. This may indicate that only small amounts of PBP 2a were present. Other published studies of the MRSA-Screen have shown a sensitivity of 97-100% and specificity of 99.2-100% (11-15). Two reviewed studies reported that, the sensitivity of the (MRSA-Screen) assay could be improved by performing the agglutination assay with isolates previously exposed to methicillin (11,16). This is consistent with the hypothesis that the *mecA* gene was present but not fully expressed and the exposure to methicillin induces the PBP 2a production. Testing after methicillin exposure was not assessed in this study, but, would be feasible and easy to incorporate into common laboratory procedures for identifying Staphylococci.

The MRSA-Screen assay is a very simple test to perform. Having to boil the organisms in a micro-centrifuge tube is inconvenient, although one study used a hot block for this step (12), which may be easier. No purchasing of any materials or equipment is required, unless using a hot block. MRSA-Screen test reagent cost is approximately $7:50 per test. This assay would be quick to establish in a standard microbiology laboratory.

A large loopful of organism is required for the MRSA-Screen, which may delay testing if direct plating only yields several colonies. A negative control is included in this assay, but, not a positive control. Tests can be performed singly or in batches.

VelogeneTM

Other studies of the VelogeneTM have shown a sensitivity of 98.5-100% and specificity of 100% (15,17-20). The Velogene™ assay requires a moderate level of technical skill to perform the testing, but this is not a problem once the method is established. More hands-on time is required than the MRSA-Screen as there are more steps, but other tasks can be performed between each one. Although the Velogene™ method involves more technical time and skill than the MRSA-Screen it gave more sensitive results in this study.

The purchase of two very precise hot blocks is required as well as the option of a micro-titer Plate Reader capable of measuring absorbance at 650nm to perform the Velogene™ assay. All tests were read visually in this study.

A very small loopful of organism is required for this assay, reducing the need and time involved in sub culturing the organism. No controls were included in the Velogene™ kit. Having a positive and negative result in each batch proved useful for comparison of blue colour development. Inclusion of control organisms in each batch would increase the cost of each patient test. The use of a spectrophotometer may overcome this problem. Reagent costs are approximately $16 per test for this assay. Testing can be performed singly or in batches.

The reagents in the Velogene™ kit, MRSA Lysis Reagent and MRSA Cycle Reagent, are stable for 14 and 10 days respectively, once reconstituted. Hence, 16 tests would need to be performed within the 10 days or 24 within 14 days to ensure that there was no wastage. This may be a problem for some laboratories. The reconstituted reagents are unable to be aliquoted and frozen to prolong their shelf life.

Some hospitals or laboratories may consider the purchase of equipment and the possibility of discarding expired reagents with the Velogene™ assay compensated by the extra sensitivity gained in using this method.

Benefits of rapid methods

In New Zealand at present, because only 1.9% of *St. aureus* are resistant, infections that appear to be of staphylococcal origin are usually treated empirically with a penicillin or cephalosporin-group antibiotic. If the infection is caused by MRSA, it may at best, resolve without the benefit of active antibiotics or at worst, progress to a more serious illness or even death. For example in a study of *St. aureus* infections in New York City Hospitals, patients with MRSA infections had a higher average attributable death rate, 21% versus 8% for an MSSA infection (21). With the use of these rapid assays for the identification of MRSA, the clinician would be able to give appropriate antibiotic treatment for MRSA 1 day earlier than if current phenotypic methodologies were used. This may reduce morbidity, time off work, the need for hospital admission, the length of hospital stay (approx. $300 per day) and the risk of dying. All of these outcomes affect the patient's family as well as the patient. Earlier recognition and treatment of MRSA infections may also, therefore, reduce costs including those of, admission to Intensive Care Unit, and extra medical procedures such as surgery or laboratory tests.

In a proportion of Staphylococcal isolates, phenotypic methods give uninterpretable results for the identification of MRSA, requiring referral of the isolate to a specialist laboratory for PCR testing. There may be a delay of up to 7 days for this result, during which time the isolate may be treated empirically as methicillin-resistant. For those infections caused by MSSA, the patient would be treated unnecessarily with expensive and more toxic antibiotics (e.g. Vancomycin at $41:80/day versus $16:72/day for Flucloxacillin) and nursed in isolation unnecessarily. Therefore, a quicker turn-around time for results, if the laboratory uses a rapid MRSA test, will help to reduce these costs and inconvenience.

Although the cost of these rapid tests may inhibit them from being used routinely for MRSA screening and all St. aureus susceptibility testing, use in conjunction with phenotypic tests for suspicious isolates and targeted patients and staff members will minimise the impact that MRSA can have.

Overall, rapid tests are likely to benefit patient care and that the savings to the hospital, community and patient should more than compensate for the additional costs of these assays.

Emerging antibiotic resistance

Vancomycin is the only drug that can consistently treat MRSA (21). The rapid identification of MRSA should restrict the use of vancomycin and, hence, the emergence of resistance to this drug of last resort.

Indeed, resistance to vancomycin is now widespread in enterococci and there are now enterococci that seem to have become resistant to all currently available antibiotics. There is (justified) concern that the vancomycin resistance genes could transfer from enterococci to staphylococci. Transfer has already been shown to occur under laboratory conditions (4). MRSA isolates with reduced susceptibility to vancomycin have recently been reported in several countries, including Japan, USA, France, Hong Kong and Scotland (22).

Recommendation of further studies

1. To enable these assays to be more extensively used in a routine medical microbiology laboratory further testing could be done on their performance on isolates taken directly from selective agar such as Mannitol Salt Agar with and without Oxacillin or Methicillin. If this testing could be done it would increase the turn-around time for MRSA screens on staff, contacts or hospital transfer patients.

2. One study (11) reported that the MRSA-Screen assay could be improved by performing the agglutination assay with isolates previously exposed to methicillin. Time and kit-size restraints did not allow for retesting the isolates after exposure to methicillin or oxacillin. Hence, a study of this factor may show that the sensitivity of the assay is higher.

3. The isolates that gave discrepant results had oxacillin MIC's of between and including 0.5 and 2 mg/l. As a relatively small number of these isolates of St. aureus was tested in this study a more in-depth analysis with a larger study-group would be recommended. This would indicate if there is problem with these isolates or that the discrepancies were statistically insignificant.

Conclusions

1. This study showed that the MRSA-Screen and Velogene™ assays are highly specific and sensitive especially when testing St. aureus isolates with Oxacillin MIC's of <0.5 and >2 mg/l.

2. Negative results obtained on St. aureus isolates with oxacillin MIC's of and including 0.5 to 2.0 mg/l with these assays should be confirmed by PCR detection of the mecA gene.

3. Either of these assays can easily be used in standard microbiology laboratories.

4. The use of the MRSA-Screen or the Velogene™ assay will allow more rapid communication of methicillin susceptibility results to clinicians which potentially could reduce mortality and morbidity, shorten length of stay in hospital, reduce both financial and personal costs, reduce the unnecessary use of Vancomycin and delay emerging antibiotic resistance.

Acknowledgments

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EXAMINATION LIFTOUT

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QTA Regulations

QTA Examination Application Form

NZIMLS Membership Application Form

The New Zealand Institute of Medical Laboratory Science offers to medical laboratory assistants the qualifications known as the Certificate of Qualified Technical Assistant (QTA) and to medical laboratory scientists the qualification known as Fellowship.

All correspondence relating to Fellowship and the QTA examinations should be addressed to:

Executive Officer
NZIMLS
P O Box 505
Rangiora
Tel: 03 313 4761
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FELLOWSHIP BY EXAMINATION
PART ONE

INSTRUCTIONS TO CANDIDATES

The examination is offered in:

Clinical Biochemistry	Clinical Microbiology
Haematology	Transfusion Science
Histology	Medical Cytology
Cytogenetics	Immunology
Virology	

EXAMINATION DATE: **13th & 14th November 2002**

1. Candidates must complete the application form and forward this, complete with examination fees, to the Executive Officer of the Institute before the closing date. No late applications will be accepted.

2. Candidates must be financial members of the NZIMLS at the time of application and examination.

3. The results of the examination will be announced by the NZIMLS. Successful candidates will be awarded the NZIMLS Fellowship by examination Part 1 Certificate in the appropriate discipline.

4. The candidate's script will be returned upon receipt of a written request by the candidate. No copy will be retained and no correspondence relating to the marking of the script will be entered into.

5. Candidates who have disabilities or injuries at the time of the examination may request the Fellowship Committee of the NZIMLS to allow a scribe. Enquires should be made to the Executive Officer of the NZIMLS.

6. The examination fee of $650 (GST inclusive) must be paid in full at the time of application.

7. Please refer to the Fellowship Regulations Section 3 for further information.

INFORMATION FOR CANDIDATES

Candidates should demonstrate an ability to integrate and apply knowledge. They should have an overall breadth and depth of knowledge of their discipline and be aware of the current literature.

Because Medical Laboratory Science is rapidly changing it is considered not feasible to set any syllabi.

The examination will consist of two, three hour written papers. The first will consist of short answer questions and the second essay type questions.

To assist candidates, a list of textbooks and recommended journals for each discipline is available from the Executive Officer of the Institute.

FELLOWSHIP BY EXAMINATION - PART ONE
APPLICATION FORM

13th & 14th November 2002

SECTION A To be completed by Candidate

Name: Mr
Mrs..... .....
Miss (Surname) (First Names)

Laboratory.....

Laboratory Address.....

.....

Examination Subject.....

Examination Fee: $650 (GST inclusive)
Full examination fee must be paid with the application

I certify that I am a member of the NZIMLS in the membership category of MEMBER and have been so for at least 2 years or am exempt as approved by the Fellowship Committee.

Signed..... Date.....

APPLICATIONS CLOSE TUESDAY 30TH APRIL 2002

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FELLOWSHIP BY EXAMINATION - PART ONE
APPLICATION FORM

13th & 14th November 2002

SECTION B **To be completed by the Charge Medical Laboratory Scientist**

I certify that the Candidate has completed at least one years post registration experience in the subject nominated for the examination.

NAME.....
(Block Letters)

Signed.....

Date.....

Please state the name and address of the person responsible for receiving the papers and supervising the examination in your laboratory.

Name.....

Laboratory Address.....

.....

.....

CERTIFICATE OF QUALIFIED TECHNICAL ASSISTANT

EXAMINATION SUBJECTS

Clinical Biochemistry
Haematology
Histological Technique
Clinical Cytology
Immunology
Transfusion Science
Transfusion Science - Blood Products
Clinical Microbiology
Clinical Mortuary Hygiene and Technique
Virology

PREREQUISITES

1. Candidates for the examination must be employed as medical laboratory assistants in an approved laboratory in New Zealand and have worked continuously in the subject for 18 months prior to the examination or accumulated not less than 18 months practical experience in the examination subject. Upon completion of two years continuous or accumulated practical experience in the subject, the certificate of Qualified Technical Assistant will be awarded.
2. Candidates who have passed a Qualified Technical Assistant examination and who wish to sit a second Qualified Technical Assistant examination must fulfil the above criteria but need only to have worked continuously or accumulated experience of one year in the examination subject.
3. Candidates must be financial members of the NZIMLS at the time of sitting the examination and be a financial member or have submitted a valid membership application form at the time of applying to sit the examination.

SYLLABUS

Copies of the syllabus are available from the Executive Officer of the NZIMLS, P O Box 505, Rangiora.

EXAMINATION

1. The examination will be held annually in New Zealand in November.
2. Candidates must complete the application form and forward this, complete with examination fees, to the Executive Officer of the NZIMLS before the closing date. No late applications will be accepted.
3. Candidates must be financial members of the NZIMLS at the time of sitting the examination.
4. The examination consists of one written paper of three hours duration. Candidates for the Clinical Cytology examination are also required to complete a practical examination.
5. To pass the examination candidates must obtain an overall mark of 50%. Clinical Cytology candi dates must pass the practical and theory examinations.
6. The results of the examinations will be announced by the NZIMLS. Successful candidates will be awarded the NZIMLS QTA Certificate in the appropriate discipline.
7. The candidate's script will be returned upon receipt of a written request by the candidate. No copy will be retained and no correspondence relating to the marking of the script will be entered into.
8. Candidates who have disabilities or injuries at the time of the examination may request the Examinations Committee of the NZIMLS to allow them a scribe. Details may be obtained from the Executive Officer of the NZIMLS.

QUALIFIED TECHNICAL ASSISTANT EXAMINATION APPLICATION FORM

6th November 2002

SECTION 1 - TO BE COMPLETED BY THE CANDIDATE

Title: Mr, Mrs, Miss, Ms Surname: _____ FirstNames: _____

Of, Laboratory: _____

Laboratory Address: _____

_____

Subject: (Haematology, Microbiology etc): _____

EXAMINATION FEE: $125 (GST Inclusive)
The full examination fee must be paid with the application.

SECTION B - TO BE COMPLETED BY THE PATHOLOGIST OR CHARGE SCIENTISTS

Date candidate commenced work in examination subject: _____

"I certify that the above candidate meets the requirements of the QTA Regulations"

Signed: _____ Designation: _____

Please state the name and address of the person responsible for receiving the papers and supervising the Examination in your laboratory or centre.

Name: _____

Laboratory Address: _____

_____

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Congratulations to Mike Lynch

It was with great pleasure that friends and colleagues of Mike Lynch read of his inclusion in the 2002 New Zealand New Year Honors List. Mike has become a member of the New Zealand Order of Merit (MNZM) in recognition of his services to community health in the South Pacific.

Mike commenced training as a Medical Laboratory Scientist in the Pathology Department at Wellington Hospital in 1955. Mike's subsequent career has been distinguished spanning a period of some 45 years in the hospital, private, commercial and academic areas of medical laboratory science.

It was, however, in teaching that Mike found greatest satisfaction and as a Tutor in Medical Laboratory Science at the Wellington Polytechnic for some ten years he made an important contribution to the education and training of many health laboratory personnel in New Zealand.

It was in this role that Mike developed an interest in technical training for the medical laboratory services of developing countries, joining the Pacific Paramedical Training Centre as Tutor- coordinator in 1985 to pursue this interest.

Since 1985 until his retirement in 2000, Mike has made a major contribution to the hospital laboratory services of the Pacific Islands and South East Asia Regions and in the honours listing is credited for "substantially improving" these services.

Mike's ability was recognized by the Western Pacific Regional Office of WHO where he was seconded for a year to develop and implement the laboratory programme in the Short and Medium term Plan in the Global Strategy for the Prevention and Control of HIV/AIDS.

During his years as Tutor-Coordinator at the Pacific Paramedical Training Centre in Wellington Mike brought a new and wider angle of vision to bear on the work of the Centre. One example of this was in the setting up of the Pacific Regional External Quality Assurance Programme. This presently involves 22 hospitals through out the Pacific Islands and South East Asia and has led to the Centre being given the status of WHO Collaborating Centre.

During the course of his career, Mike has traveled extensively throughout the Pacific and Southeast Asia working in most countries including Samoa, Tonga, Vanuatu, Fiji, Kiribati, Marshall Islands, Federated States of Micronesia, China, Vietnam, Cambodia and the Philippines.

Mike has shown untiring enthusiasm and initiative in development assistance to the health laboratory services of the Pacific Islands. This, together with his personal qualities, has made him a respected figure in the profession and has reflected well on the NZIMLS at an international level. In recognition of his services to Medical Laboratory Technology in New Zealand and the Pacific Region, Mike was awarded Life Membership of the NZIMLS in 2000 .

Dr Ron Mackenzie, Chairman, Pacific Paramedical Training Centre, Wellington Hospital

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Answers to HSIG journal based learning questionnaire

1. True
2. True
3. True
4. False
5. False
6. False
7. True
8. False
9. False
10. True
11. False
12. True
13. False
14. True
15. True
16. True
17. True
18. False
19. True
20. True
21. True
22. True

New Zealand Institute of
Medical
Laboratory
Science

Council News

March 2002

Welcome to 2002 .

We hope everyone had a safe and enjoyable festive season.

CPD programme

The review of the claims for the 2000-2001 period is now completed. Those who participated in the first year should have by now received back the totals for the first year of the programme. As the claim sheets were received a little slower than we expected the turn around time has been longer than we had hoped. This will improve as provision of CPD claims become a regular part of the yearly activities. Remember that your claims for the 2001-2002 year are due in after the 1st April this year. Tally sheets and CPD information is available through the NZIMLS webpage at www.nzimls.co.nz

The Health Professionals Competency Assurance Bill

Council met with the Medical Laboratory Technologist's Board on 23rd November to discuss the upcoming HPCA Bill. Marilyn Goddard from the Ministry of Health, outlined the Bill and clarified several issues regarding the "Licensed Tasks" and "Scopes of Practice". The HPCA Bill has been drafted to protect the public against injury or harm that could result from the actions of an incompetent Medical Laboratory Scientist. From the meeting it is likely that the performance of certain laboratory tests will be restricted to those licensed within a "Scope of practice". To be licensed to perform these tasks, each individual will be required to provide documentary proof of competence. The "Scopes of Practice" are yet to be established but will need to be determined in the near future. Training and competency records for staff, (already required for IANZ accreditation) and the CPD program, look set to play an important part in providing the evidence necessary to support annual licensing. 2002 looks set to be a busy year, with big changes to the regulation of our profession likely to take effect in the future (although it is election year!!).

NZIMLS in association with Healthlab Otago presents :-

South Island Seminar - Lake Tekapo

Saturday 16th March 2002

A day of short presentations and a long night of fun!

For more info: Cathy Stevens
CathyS@healthotago.co.nz

Please note Please note Please note Please note

Change of date

Symphony of Science !!!

Annual Scientific Meeting

Te Papa

Wellington

7 - 10th August

Have you checked out our website recently??????

www.nzimls.org.nz

NICE WEEKEND

Wairakei Resort Hotel

19 - 21 April 2002

A Transfusion Science Education Opportunity

Registrations to: Grant Bush
Transfusion Lab
Medlab BOP
P O Box 130
Tauranga

Please note that registration is limited to 50 participants and preference will be given to NZIMLS members

Thirteenth Annual

NICE WEEKEND

A Transfusion Science educational opportunity organised by the TSSIG

at Wairakei on 19-21 April 2002

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

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

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

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

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

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

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

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

BSIG SEMINAR

CHRISTCHURCH

20 JULY 2002

THIS YEAR'S TOPIC OF DISCUSSION

CANCER MARKERS

Why mark a Tumor?

Because we can sir!

BSIG SEMINAR

Venue: Copthorne Hotel, Durham Street, Christchurch
Date: Saturday 20 July 2002
Seminar Start Time: 1000 hrs with registration from 0900 hrs

Registration Form

First Name:	
Surname:	
Laboratory:	
Contact Phone No:	
Email Address:	

Proffered Papers

(any biochemical topic)

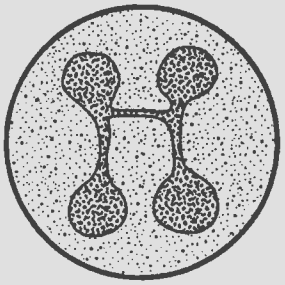
Title of Paper:	
Equipment Required:	**Overhead Projector** **Slide Projector** **Powerpoint** **Presentation inquiries to: Chris Sies** **Canterbury Health Laboratories** **Tel: (03) 3640332** **Email:** Chris.Sies@cdhb.govt.nz

Registration Fee

(includes lunch, morning and afternoon tea)

NZIMLS Member	**$45.00**		**$**
Non Member	**$60.00**		**$**
Dinner	**$45.00**		**$**
Please return this form and payment to: Fran van Til **PO Box 505 Rangiora** **Please make cheques payable to: NZIMLS BSIG Seminar** **Direct any inquiries to: Jan Deans, Canterbury Health Laboratories** **Tel: (03) 3640484 or Email:** Jan.Deans@cdhb.govt.nz **Sandy Woods, Canterbury Health Laboratories** **Tel: (03) 3640332 or Email:** Sandy.Woods@cdhb.govt.nz		**Total:**	**$**

Accommodation to be arranged individually
Registration and payment to be forwarded by 5 July 2002



✳ Attention Senior Scientists ✳

QUALITY ISSUES IN HAEMATOLOGY

Presentations & Interactive Discussion

Quality Assurance • Quality Control • Statistics • Method Evaluations
including Haemostasis

Ellerslie Motor Inn, Auckland, May 2002
Registration Forms & more details available from Haematology Managers now
Closing Date April 19th; Limited numbers apply

HSIG journal based learning - questionnaire

White blood cell and platelet counting performance by hematology analyzers: a critical evaluation. Bernard W Steele, Niou-Ching Wu, Clarence Whitcomb. Laboratory Haematology. 2001; 7:255-266

Please circle the correct response

1. Three of the four analysers have a positive bias for platelets.
TRUE / FALSE

2. This study suggests an analyser cutoff value of 15 x109/L for platelet transfusions.
TRUE / FALSE

3. Platelet Counts can be elevated in the presence of cellular fragments.
TRUE / FALSE

4. With reference to the analysers assessed, unlysed red blood cells /nucleated red cells do not affect the accuracy of the white cell count.
TRUE / FALSE

5. Manufacturers develop algorithms that compare results to reference methods, all of which are manual techniques.
TRUE / FALSE

6. Instrument flagging of low platelet counts has led to reduced delays in reporting results.
TRUE / FALSE

7. The reference method for flow cytometry platelet counting was published last year in the American Journal of Clinical Pathology.
TRUE / FALSE

8. A group of patients with platelet counts between 150 and 400 was used as the control group for this study.
TRUE / FALSE

9. One group of patient samples was used to test the ability of the algorithms to handle red cell lysis problems that may interfere with platelet counts.
TRUE / FALSE

10. The study required one group of samples to pass three screening algorithms for thalassemia.
TRUE / FALSE

11. The authors found low level impedance platelet counts on the Cell-Dyn 4000 to be reliable.
TRUE / FALSE

12. The identification of platelets is assured by the use of both CD41-FITC and CD61-FITC.
TRUE / FALSE

13. In flow cytometry, red blood cells are not discriminated from platelets by fluorescence or light scatter signals.
TRUE / FALSE

14. The Gen(S algorithm flags all samples with platelet counts less than 20 x109/L.
TRUE / FALSE

15. ADVIA 120 results with a platelet noise flag would have been verified by blood film examination in this study.
TRUE / FALSE

16. None of the flagged platelet results from the four analysers were shown to be outliers when compared to the data obtained from the flow cytometer.
TRUE / FALSE

17. When the reference value is set at 10 x109/L and the analyser flag value is set at 15, 18-20 of the 29-31 patients tested were correctly identified as not requiring platelet transfusions.
TRUE / FALSE

18. The ADVIA 120 flags for nucleated red cells and corrects the white cell count for them automatically.

TRUE / FALSE

19. High-mean platelet volumes may cause low platelet counts to be flagged.

TRUE / FALSE

20. This study was supported financially by one of the manufacturers of analysers used in this study.

TRUE / FALSE

21. Reducing the analyser cutoff for platelet transfusion to 15 x109/L from 20 x109/L, the number of unnecessary transfusions would be reduced by 55%.

TRUE / FALSE

22. Regression and correlation statistics were very similar between the four analysers.

TRUE / FALSE

If you wish to receive a copy of this article by mail please contact Tania Isobel, Haematology, LabPlus, Auckland District Health Board

Phone (09) 3074949 ext. 5995

Answers on page.....31

Microbiology

S.I.G. Meeting

CHRISTCHURCH

Friday 10th and Saturday 11th May 2002

SEMINAR: Friday 10th May 5.30 - 8pm
Venue: Seminar Room, 2nd floor, Canterbury Health Laboratories
Topics: Parasitology, Dysmorphic Red Blood Cells in Urine
The content has not fully been decided on for this seminar, but will include pizzas and drinks!

SIG MEETING: Saturday 11th May 9.15am - 5pm

Venue: Blackwell Room, Copthorne Hotel, Durham Street, Christchurch.

Schedule:

9.15am	Registration and morning tea
10.00am	Presentations Case studies, reviews, results of trials, etc. Talks need to be 5-15 minutes. We will have access to overheads, slides and computer/data projector for the presentations. Lunch and afternoon tea will be included.

Dinner: 7pm, Copthorne Hotel. This promises to be a fun night!

- This is the first year this meeting has been held in the South Island, so we expect to see lots of delegates from South Island labs!
- An excellent opportunity for people in the North Island labs to visit our lovely city - lots to see & do.
- We have some very good presentations already lined up - a good mix for all interests.
- For registration forms contact:

Julie Vincent
Canterbury Health Labs
phone (03) 3640300 ext 89826
julie v@cdhb.govt.nz

Book reviews

Haemoglobinopathy Diagnosis. Author: Barbara Bain, MBBS, FRACP, FRCPath. Published by Blackwell Science, 2001.

This compact book (260 pages including index) is a valued addition to the laboratory library, especially if routine screening for haemoglobinopathies is undertaken. The chapters are well laid out, in similar form to other Barbara Bain books ("Blood Cells - A Practical Guide" and "Bone Marrow Pathology"), with frequent use of coloured background tables and diagrams and photographs. Following the initial chapter on haemoglobin structure, function and genetics, the practical topics covered are; laboratory detection techniques, the thalassaemias α, β, δ and γ, HbS and its interactions with other variant haemoglobins and thalassaemias, other significant haemoglobinopathies and a chapter on acquired abnormalities of globin chain synthesis and haemoglobin structure. The penultimate chapter addresses the organisation of a haemoglobinopathy service and has flow charts for suggested testing. The last chapter is solely a self assessment exercise of test cases employing test results, diagrams and photographs of blood films and electrophoretic strips.

The information given in this book is concise, (though in some areas more descriptive), and has proved very helpful with practical queries in our laboratory. Besides the final chapter, there is a self-assessment exercise in the form of true/false answers at the end of each chapter, and also a list of suggested further reading sources. The current cost of this first edition is NZ$180.60 and, I consider, well worth it.

Reviewed by Jacquie Case, Special Haematology, Middlemore Hospital, Auckland

The Thalassaemia Syndromes, 4th ed. Authors: DJ Weatherall and JB Clegg. Published by Blackwell Science, 2001

Written and edited by D.J. Weatherall and J.B. Clegg, both of the molecular haematology unit, Institute of Molecular Medicine at the University of Oxford. The fourth edition of "The Thalassaemia Syndromes" was written some 20 years after the publication of the third edition. As more and more analysis was being done at the DNA level there was a need for review of the genetic and phenotypic variability of the thalassaemias. The authors could not find an appropriate time to produce a fourth edition as the information seemed out of date as soon as it was written. More recently the development in the thalassaemia field has slowed considerably and the authors thought it fitting to update the previous edition.

Using an extensive list of references, "The Thalassaemia Syndromes" covers the following topics in depth:

- Historical background of the thalassaemias explaining the different discoveries that led to our current understanding of the thalassaemia syndromes.
- Biology of the thalassaemias - human haemoglobins, the classification of thalassaemia and the relationship to other inherited diseases of haemoglobin; molecular pathology and pathophysiology of the thalassaemias; and the world distribution and population genetics.
- Clinical features of thalassaemia including beta thalassaemia; delta beta thalassaemia; hereditary persistence of foetal haemoglobin; alpha thalassaemia; the thalassaemias in association with the structural haemoglobin variants; syndromes of alpha thalassaemia and mental retardation; and thalassaemia intermedia
- Diagnosis and management of thalassaemia - avoidance and population control, management and laboratory diagnosis.
- The future - approaches to screening, treatment, population control, and definitive treatments (bone marrow transplantation).

This book is an excellent reference textbook for use in the laboratory and also provides comprehensive clinical information for haematologists. It is aimed at specialists in haematology, in both clinical and laboratory settings, and in particular areas where thalassaemia is common either in the native population or in immigrant communities. Considerable emphasis is placed on the molecular pathology and reflects the significant advances made in the last few years. It can be somewhat bewildering to those with a limited understanding of molecular genetics but each chapter starts with the basics then leads into the more complex sections. This textbook has 864 pages including 284 illustrations and is currently priced at NZ$766.00.

Reviewed by Lisa Rae, Special Haematology, Diagnostic Medlab, Auckland

Winning the Publications Game, 2nd ed. Author: Tim Albert. Published by Radcliffe Medical Press, Oxford, 2000

Many books and articles have been written on how to write scientific papers for biomedical journals. Unfortunately, the majority of these are written in a dry, boring manner. This book by Tim Albert, a former journalist and now a trainer who specialises in teaching health professionals how to write and edit scientific articles, is the exception. It is written in an easy readable, fun style. In his preface Tim writes, "This book stems from my conviction that writing a scientific paper is not as hard as many people are led to believe".

In the following chapters the book takes the reader through ten fundamental steps of writing a scientific paper. (A brief account of these ten steps are elaborated on in the Leading Article by Tim Albert, "Write a scientific article - the easy way", in this issue of the Journal). All chapters contain 'pearls of wisdom' for anyone writing scientific papers, from the novice to the more experienced. Included are many Tables summarising briefly, but succinctly, the salient points.

Having published many scientific articles, I still thought that writing was hard, until I read Tim's book. Every time now when I put pen to paper (or two fingers on the PC key board) I automatically reach for Tim's book. I thoroughly recommend this book for medical laboratory scientists who are contemplating writing scientific articles (preferably for our Journal!), and especially members of our profession taking the Fellowship part 2 by treatise or thesis. This book should be in every library.

Reviewed by Rob Siebers, Editor, New Zealand Journal of Medical Laboratory Science

New products and services

VIDAS CMV IgG Avidity improves the exclusion diagnosis of recent primary infection in pregancy.

BIOMERIEUX has released the VIDAS CMV IgG Avidity parameter to complement its existing range of 58 VIDAS assays available in Australia and New Zealand.

The rapid diagnosis of CMV infection in pregnant women is important as it is one of the most common causes of congenital infection in industrialized countries. Serological testing is often used in diagnosis, but demonstrating seroconversion with CMV IgG takes time and delays diagnosis. CMV IgM testing is also problematic - if this result is positive the question remains: "Is it a primary infection?"

VIDAS CMV IgG Avidity helps a laboratory answer this question with the reliable exclusion of a primary infection (less than 3 months duration). The test differentiates between the low avidity antibodies associated with recent infection and the high avidity antibodies seen with less recent infections.

VIDAS is a precise, reliable and compact system that is available around the clock with no required start-up or wait time. It is easily adapted to diverse laboratory situations. For these reasons, VIDAS is a testing solution that is relied upon by more than 12 000 small, medium, and large laboratories around the world.

For more information on VIDAS or VIDAS CMV IgG Avidity parameter please contact:

BIOMERIEUX Australia Pty Ltd
Tel: 1 800 333 421
Fax: 1 800 065 421
Email: clinical@biomerieux.com.au
Web: www.biomerieux.com.au

Molecular biology technology.

Thermo Labsystems KingFisher offers a new possibility to automate almost any purification or isolation procedure in the field of molecular biology. This technology based on the paramagnetic particles is a useful tool helping small and medium sized laboratories to eliminate time consuming precipitation or column purification steps. With KingFisher, pipette the starting material and reagents into the microplate or tube strip and start the process. The result is high purity molecules or cells in 10-30 minutes for the desired downstream application.

Also available is the KingFisher genomic DNA purification kit, KingFisher

mRNA purification kit and cross-contamination free processing.

For further details contact Medica Pacifica Ltd 0800 106 100 or email: info@medica.co.nz

Index to Volume 55, 2001

Otago University BMLSc graduation, 2001

Rebecca Wilson, Jim le Grice award, top clinical biochemistry student receiving award from Trevor Rollinson

Karen Hall, top overall student receiving award from Dr. C Lovell-Smith

Philip Wakem, Masters degree in medical laboratory science (MMLSc)

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About MLS

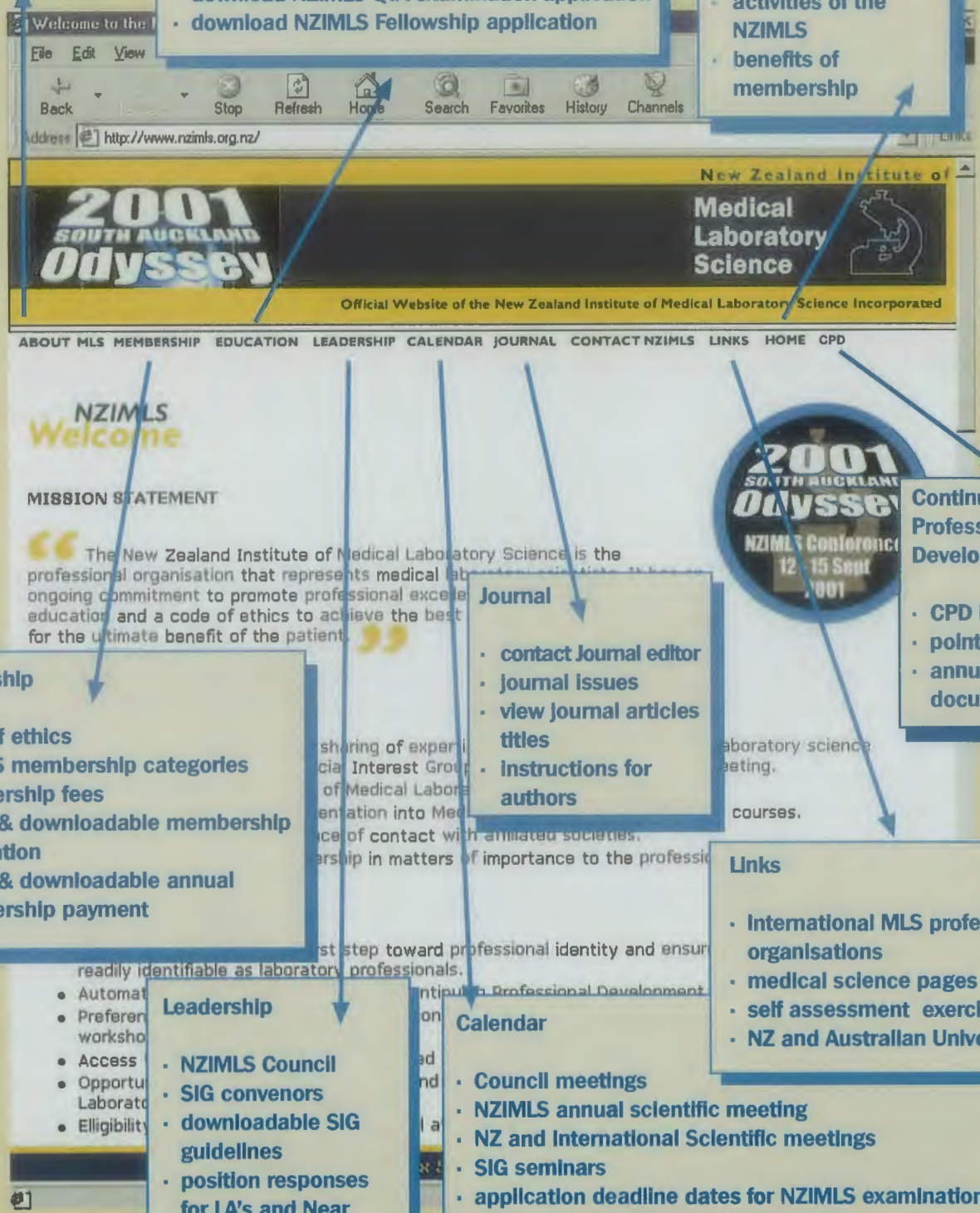
- career information
- MLS disciplines
- ASM information

Education

- MLS degree programmes
- download NZIMLS QTA examination application
- download NZIMLS Fellowship application

Home

- mission statement
- activities of the NZIMLS
- benefits of membership



Membership

- code of ethics
- NZIMLS membership categories
- membership fees
- online & downloadable membership application
- online & downloadable annual membership payment

Journal

- contact Journal editor
- journal issues
- view journal articles titles
- instructions for authors

Continuing Professional Development

- CPD information
- points allocation
- annual return documentation

Links

- International MLS professional organisations
- medical science pages
- self assessment exercises
- NZ and Australian Universities

Leadership

- NZIMLS Council
- SIG convenors
- downloadable SIG guidelines
- position responses for LA's and Near Patient Testing.

Calendar

- Council meetings
- NZIMLS annual scientific meeting
- NZ and International Scientific meetings
- SIG seminars
- application deadline dates for NZIMLS examinations
- Fellowship and QTA examination dates

MODULAR ANALYTICS <E>

Roche

Heterogenous Immunoassay Testing

One you can rely on

E170

Simply Modular

Roche Diagnostics New Zealand Ltd
15 Rakino Way, Mt Wellington, Auckland
Tel: +64 (9) 276 4157 Fax: +64 (9) 276 8917