New Zealand Institute of
Medical
Laboratory
Science

ISSN 1171-0195 Volume 64 Number 3 November 2010

Established in 1946

New Zealand Journal of Medical Laboratory Science

Official Publication of the New Zealand Institute of Medical Laboratory Science Incorporated

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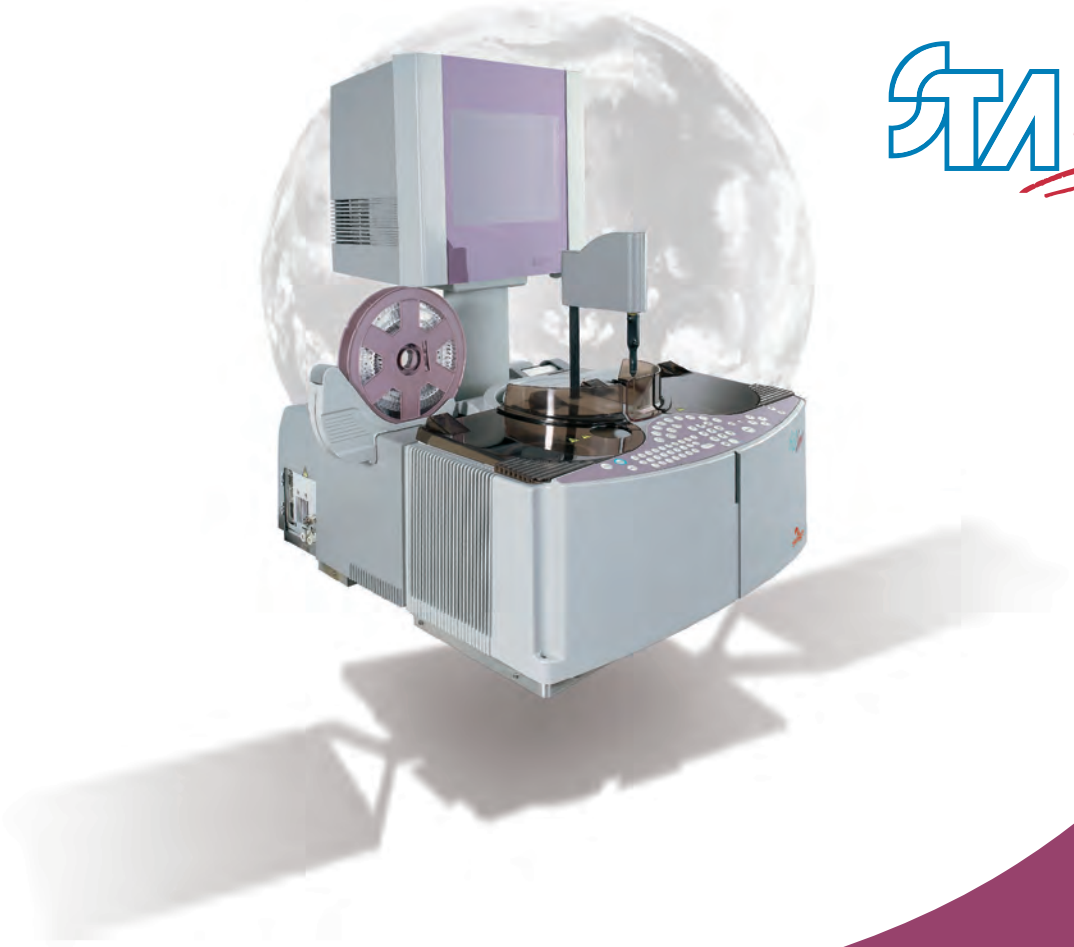

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Brief instructions to authors

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New Zealand Journal of
Medical Laboratory Science
Volume 64 Number 3
November 2010
ISSN 1171-0195

TH Pullar Memorial Address

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Scientific letter

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New Zealand Institute of Medical Laboratory Science

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Successful applicants will be required to provide a full written report on return which will be published in the Journal. If not intended to publish elsewhere, successful applicants will be required to submit their study results for consideration by the New Zealand Journal of Medical Laboratory Science.

Congratulations to Sandy Woods from the Biochemistry Department at Canterbury Health Laboratories who is the first recipient of the Barrie Edwards and Rod Kennedy Scholarship. Sandy will attend the South Pacific Congress in Brisbane next year where she intends to give an oral scientific presentation and prepare a conference report and paper for the Journal.

In this issue

Jing Fung Kee examined the usefulness of Glut-1 as a tool to distinguish between benign and malignant mesothelial cells in fluid cytology. He found that Glut-1 positivity was seen in 82.4% of malignant mesotheliomas and concludes that Glut-1 immunohistochemistry is a useful adjunct to routine fluid cytology in patients with possible mesothelioma. Glut-1 is best interpreted together with other markers including epithelial membrane antigen and desmin.

Richard Omoregie and colleagues determined the prevalence of extended spectrum ß-lactamase producing Gram-negative bacteria (ESBL) from surgical wound and blood stream infections in a teaching hospital in Nigeria, where third generation cephalosporins are used as blind therapy. They found an overall prevalence of 2.7% of ESBL producing Gram-negative bacteria and isolates from blood were 3 to 22-fold more likely to produce ESBL. Prudent use of antibiotics is advised.

Andrew Stewart reports on the characterisation of subtypes of HLA-B27 in samples that had been referred for HLA-B27 testing due to clinical symptoms characteristic of spondylarthropathies and anterior uveitis. He found that the subtypes reflected the HLA-B27 subtypes found in previous studies of the different ethnicities involved. This study was part of Andrew's successful NZIMLS Fellowship examination.

Macrocytosis is a common finding in the hospital and community setting, generally attributed to alcoholism, vitamin B-12 and folate deficiencies and certain medications. Only few studies have reported macrocytosis in chronic obstructive pulmonary disease (COPD). Francis Wu and colleagues from Taiwan and New Zealand retrospectively determined the prevalence of increased mean red cell volume in COPD in an out-patient setting. They found a macrocytosis prevalence of 23.3% in this setting and conclude that physicians and laboratory scientists have to be aware that a raised MCV in COPD may be due to factors other than the usual known factors associated with macrocytosis.

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TH Pullar Memorial Address
Believe in yourself, and anything is possible

Christine Pry, Country Manager, Abbott Diagnostics, New Zealand

The year is 1981. Please imagine a young woman, 17 years old, who has just finished her 7th form year at Kelston Girls High School. (That's Year 13 for all the young people here today). She has a big decision ahead of her, does she go out and find a job or go to university?

The subjects she enjoyed most were chemistry, biology and physics – so clearly she's interested in pursuing something in the field of science. She's persuaded to apply to become a laboratory technologist by her favourite uncle who's working at Auckland Hospital at the time – in charge of the Microbiology Department there. His name was Graham Cameron and he seemed to genuinely love his job - so she decides to apply.

She finally receives a letter in the mail inviting her to attend an interview for a position as a trainee laboratory technologist at National Women's Hospital. The day of the interview finally arrives. She walks into a room – trying hard to steady her nerves, and is asked to sit down in front of a row of men (who seemed to look very old and serious). They proceed to fire questions at her, regarding her interest in becoming a lab scientist – it is all rather stressful. She leaves the interview hoping it has gone well, but feeling extremely nervous. Finally, some weeks later, a formal letter arrives. She runs to her room to open it in private. She lets out a huge scream of excitement when she realizes she has been accepted as a trainee into the New Zealand Certificate of Science Paramedical course.

At that time, this young woman would never have dreamt she would be standing in front of you now and being given the honour of presenting the TH Pullar Address at the opening of a New Zealand Medical Laboratory Science Conference. I must admit, I was surprised when I received a letter from Fran a few months ago – asking me to speak at this year's conference. I had to read the letter a couple of times because I kept thinking "they've got it wrong." I remember calling my husband and telling him the news and his reply was "well, perhaps they want you to help organize a guest speaker from overseas." I assured him it truly was me they were referring to– and I must say, it is a great honour to be standing here before you.

My message today is simple: "Believe in yourself, and anything is possible." That's the kiwi spirit. I'm also going to talk about how quickly our society is changing and the implications of this for our industry.

For a very small country New Zealand can be proud of many people. Famous musicians such as Tim and Neil Finn, renowned artists such as Charles Goldie, sports people like Peter Snell, John Walker, the All Blacks – well for the moment anyway! We have famous film directors such as Sir Peter Jackson and world class aviators such as Jean Batton. Sir Edmund Hillary was, of course, the first to conquer Everest. We were the first country in the world to allow women to vote - thanks largely to the work of Kate Shepherd. New Zealand was the first country to introduce the eight hour working day. We have celebrated this every day (on Labour Day) since 1899. We were the first country to have its three top positions of power held simultaneously by women: the Prime Minister (Helen Clark), the Governor General (Dame Silvia Cartwright), and the Chief Justice (Sian Elias). I find it interesting that Sian Elias earns more than John Key!

For a small country, we have much to be proud of. Of course we all know that it was Richard Pearse from Temuka who first invented the airplane. And we all know, this was several months before the Wright Brothers. I'll never forget the day my son came home from school after doing a project on Richard Pearse and started explaining the achievements of this famous kiwi at the dinner table. It took some time (and help from the internet) for him to convince my American husband that this person actually existed – as coming from the States, Terry had never heard of him.

Richard Pearse was a reclusive New Zealand farmer, a self taught inventor who, on a warm day in 1903, climbed into a self built monoplane and flew 140 metres before crashing into a gorse bush at the end of his Waitohi property. Imagine the exhilaration he must have felt as the aircraft flew for the first time! Sadly, he died in obscurity and his amazing achievements were largely forgotten until one of his prototype airplanes were discovered in a Christchurch garage. His death was clouded by the controversy over whether or not he flew before the fully documented Wright brothers flights. All this aside, we should acknowledge Pearse's achievements were even more remarkable in that, unlike the Wright brothers who employed skilled engineers and later enjoyed the luxury of Government sponsorship, Pearse managed to get airborne with no technical training and very little resource. He relied on practical ingenuity and "trial and error" innovation to design, finance and build everything himself.

There are also many scientists born and educated in New Zealand who have achieved great things. Now let me take you back to 1871. A boy was born into a large family of seven boys and five girls. He attended Nelson Collegiate School and received a scholarship to attend Victoria University. In his early work as a research scientist he discovered the concept of radioactive half life and went on to win a Nobel Prize in chemistry in 1908 – for his work on the disintegration of the elements and chemistry of radioactive substances. He actually performed his most famous work after he received this prize and is widely credited with first splitting the atom in 1917.

Of course, this was Ernest Rutherford – probably our most famous scientist. As a nation we are so proud of Rutherford. We have schools and streets named after him, his picture is on our $100 bill and there is even a crater on the moon named in his honour. Isn't it ironic that it was a New Zealander who was instrumental in convening the Manhattan Project – which developed the first nuclear weapons when we are the first country to become officially "Nuclear Free."

After Rutherford it was to be another 92 years before another New Zealand educated person was to win a Nobel Prize. This was Alan MacDiarmid who was instrumental in the discovery of conductive polymers – plastics that conduct electricity just like metals. This has already led to innovations in LED screens in the latest televisions and cell phones. Like many of our parents (or grandparents) Alan was born at the time of the Great Depression – in the late 1920's. He grew up in Masterton and was the youngest of five children. It was common to go to school barefoot. The family bathed just once a week and the younger children used the bath water after the older children. These tough times imbued Alan with an unshakeable work ethic which he reflected on after receiving his Nobel Prize in 2000. He was extremely proud to be a kiwi.

Alan was the epitome of a research scientist – embodying total dedication to his subject. He kept a writing pad beside his bed to jot down ideas that come in his most productive thinking times– such as dozing or when in the shower. Science is people, singular or collective, discussing ideas with colleagues. If you have very good

people working not for you, but with you, then the chances of achieving great things are high.

Of course we can't all be a Rutherford or a Pearse - but my message remains – if you believe in yourself, anything is possible. The New Zealand pathology industry has gone through so many changes since I first started as a trainee at National Women's Hospital. Looking back, this was a great time in my life. I had the opportunity to work with so many knowledgeable and inspiring colleagues, many of whom are still in the industry. We had lots of laughs working in the laboratory and thought it was funny when someone had an accident mouth pipetting – until we realized what it was doing to the inside of their mouth! The strange thing is we also thought it was quite normal for body parts and small perfectly formed foetuses to be stored in formalin around the lab. We worked closely with our colleagues and formed life-long friendships.

It was during the early these years in the early 80's that people first started talking about HIV and I remember feeling a bit apprehensive about working with blood samples – and having no idea of their HIV status. There was also a real fear in the community – can you imagine delaying a life-saving surgery or being afraid to receive or donate blood for fear of contracting HIV? It seems unbelievable today, but twenty-five years ago this was the reality as the world struggled to keep the deadly virus out of the blood supply.

In 1985 an estimated one in 100 blood transfusions was infected with the HIV virus in some United States cities. Twenty-five years later - this risk is now minimal and millions of patients are safely given blood transfusions each year. On March 2, 1985, the U.S. Food and Drug Administration (FDA) approved the first-ever diagnostic test to screen blood donors for antibodies to HIV. This first test was developed by Abbott – the company I was to join five years later.

The theme of this year's conference is "Diversity in Science." When I think back on my experiences with laboratory science it certainly has been diverse. But when you think about it we live in a very diverse society. Did you know the top ten "in demand" jobs in 2010 did not even exist in 2004? We are currently preparing students for jobs that don't yet exist, using technologies that haven't been invented, in order to solve problems – we don't even know what the problems are yet.

We are living in exponential times. Just 10 years ago there were only 360 million people around the world, regularly using the internet. Today, this number has risen to almost 2 billion people – almost a third of the world's population. In 2006 there were over 25 billion indexed pages available on Google, with 3 million searches per day. Now there are an almost infinite number of pages on Google, with over 400 million searches per day! Not only that, there are now 119 different languages that you can search in. It makes you ask the question "Who did these people ask before Google?"

If Richard Pearse was able to invent the airplane without this resource, imagine what he might have done now. The first commercial text message was sent in December 1992. Today, the number of text messages received and sent everyday exceeds the total population of the planet – and changing the way we spell words.

Think about this, if you are one in a million, and you live in China, there are 1300 other people, just like you! China will soon become the largest English speaking country in the world. The 25% of India's population with the highest IQ's is greater than the total population of the United States. In translation, this means India has more honours kids than America has kids!

Today's student will have 10 to 14 jobs, by the age of 38. One in four workers has been with their employer for less than one year. One in two has been there less than five years. Did you know one out of eight couples married in the US last year met on-line. It took 38 years before the first radio was sold – to reach a market audience of 50 million. TV's took just 13 years, the internet took four years, i-POD's took 3 years and in just 38 days Apple has sold one million i-PADs.

The amount of new technical information is doubling every two years. For students starting a four year technical degree course this means that half of what they learn in their first year of study will be outdated by the third year of study. These facts are both depressing – and exciting. For me it means the training I did in the lab 20 years ago has no bearing whatsoever on what is required today. So, I better not give up my day job. However, I feel very grateful for having had the opportunity to work in this exciting field – even if I am "on the dark side."

There have been some funny moments in my career at Abbott over the years. I remember the time I was sitting on a plane with a colleague and saying how exciting it was that we now had syphilis! The person next to me spent the rest of the flight leaning as far away from me as possible. This is really showing my age, but I remember clearly the day I got my first mobile phone. Well, it wasn't entirely mobile as it was the size of a brick, attached with a thick tight coil to the centre console of my car. I felt so important driving around with this amazing "state of the art" technology! The instruments we sold back then could be loaded into the back of your car, and off you went, around the country selling your wares. Of course the automation and size of equipment now make this impossible. Other highlights would have to be pinching free coffees from the Roche stand each year at conference!

All of this made me wonder, what would Thomas Pullar have thought of all this. From everything I have heard, it sounds like Thos was another great example of someone who had real "kiwi spirit". He dedicated his life to medicine – and was a huge influence at the time in laying down the foundations for medical laboratory science in New Zealand.

So much has happened since his time, but the exciting thing is, so much more is ahead of us. Looking into the future we have every opportunity to make a difference. When I was asked to do this speech I wanted to try and make it inspiring and uplifting. It's hard, however, not to acknowledge we are experiencing difficult times within our industry. Last year we were all coming to terms with laboratories closing down, new ones opening up. We watched highly qualified people lose their jobs around the country through amalgamation and in the bid to reduce costs. This year, even as we speak, we are in the midst of industrial action – which is putting extreme pressure on all concerned.

We must remember we have been through difficult times before. We have pulled through and continue to move forward. Twenty five years ago, just before I was about to leave NWH to travel overseas, we were in the midst of industrial action due to the fact that lab technologists had not received a pay increase for five years straight. I remember this being an incredibly difficult time for everyone and I feel for all those impacted by current events.

We should never forget the value that laboratory science significantly adds to the health of patients all around the country. We know that although the laboratory only represents a small percentage of medical costs, the results produced are used to leverage 60 to 70 percent of all critical decisions – from admission to, discharge and drug therapy requirements of a patient. This is what we do – what we are passionate about. We need to work together to get this message out to the public and political domains – to demonstrate the true value of laboratory science. Each one of us needs to play our part.

The epitome of kiwi spirit is to be at the forefront of change. That makes us ideally positioned in the new environment we now operate in. Now is the time to show what real kiwi spirit is. What makes us different sets us aside from others and brings me back to my key message: "If you believe in yourself, anything is possible". It is vital for us to hold this belief when we are so immersed in a climate of unprecedented change.

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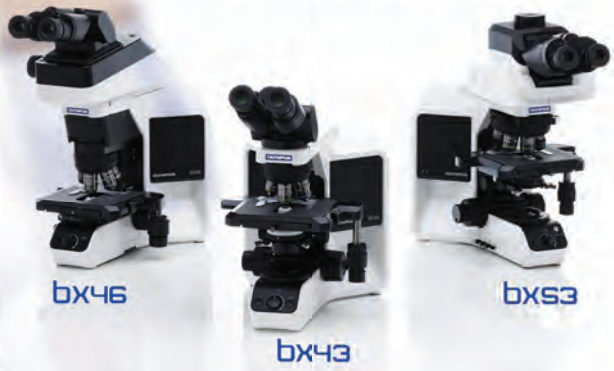

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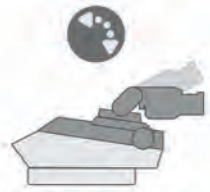

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Immunohistochemical detection of Glut-1 helps to distinguish between reactive mesothelium and malignant mesothelioma

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Abstract

Objective: The distinction between benign mesothelial cells and malignant mesothelial cells in fluid cytology is a common scenario facing pathologists. This study aims to examine the usefulness of Glut-1 as a tool in making this distinction when applied to cell block material. Glut-1 staining was interpreted in conjunction with desmin. Glut-1 staining in mesothelioma was compared to epithelial membrane antigen staining.

Methods: 34 cytology cases (17 benign and 17 mesothelioma) reported at Labplus between 2002 and 2009 were reviewed. Glut-1, desmin and epithelial membrane antigen immunohistochemistry were applied to the cell blocks. All cases in both categories had the diagnoses confirmed histologically. The staining results were then divided into negative, focal positive (<30% staining) and diffuse positive (>30% staining).

Results: Glut-1 positivity was seen in 82.4% of malignant mesotheliomas. Only one case of mesothelioma showed focal weak positivity for desmin while the other 16 cases were completely negative. All 17 cases of benign mesothelial proliferation were negative for Glut-1. Of the 17 cases of benign mesothelial proliferation, two showed focal positivity for desmin, four were negative, while the rest were all strongly positive.

Conclusion: Glut-1 immunohistochemistry was proven to be a useful adjunct to routine fluid cytology in patients with possible mesothelioma. Negative staining with Glut-1 does not rule out mesothelioma, especially when clinical suspicion is high. Glut-1 is best interpreted together with other markers including epithelial membrane antigen and desmin.

Keywords: mesothelioma, mesothelial hyperplasia, Glut-1, desmin, epithelial membrane antigen.

N Z J Med Lab Sci 2010; 64: 70-73

Introduction

The finding of atypical mesothelial cells is a common encounter in fluid cytology. However, distinguishing reactive mesothelial cells from malignant mesothelial cells is often difficult in cytology. Until recently, histochemical stains and electron microscopy were the standard tools used for the diagnosis of malignant mesothelioma. With the advent of immunohistochemistry, pathologists now rely on immunohistochemical markers as adjuncts to routine histology when it comes to diagnosing malignant mesothelioma. Unfortunately, no absolutely specific or exclusive antibodies have been yet identified for such a diagnosis. Epithelial membrane antigen (EMA) and desmin are two antibodies frequently used for the purpose. Recent literature has examined the reliability of Glut-1 in discriminating between reactive and malignant mesothelium in histological sections. This study evaluated the usefulness of Glut-1 when applied to cytology samples.

Materials and methods

Case selection

The materials for the present study were extracted from cases reported by the Labplus cytology department, Auckland, New Zealand, between 2002 and 2009. The benign mesothelial cell group comprised of six cases of pleuritis/pleural plaque, two cases of pericarditis, one case of multiloculated benign peritoneal inclusion cyst and eight cases of reactive mesothelial proliferation in the context of gynaecological malignancy staging (peritoneal washing). The malignant group was composed of 17 cases of malignant mesothelioma, one of which was sarcomatoid and one showing mixture of sarcomatoid and epithelioid patterns. The diagnosis in all cases was confirmed on the basis of conventional histopathologic features evident in subsequent hematoxylin and eosin stained tissue sections.

Immunohistochemistry

All the cases in this study had cellblocks done at the time of initial processing. In our laboratory, fluid samples were spun down into cell buttons by centrifugation. Human derived serum was added to the cell buttons and left standing for 2-3 minutes. Thrombin was then added to the mix and this produced clots containing the cells of interest. The clots then went through routine automated procedures to get paraffinised.

The aim of this study was to examine the usefulness of Glut-1 when applied to cellblock material when used in conjunction with application of EMA and desmin. The staining intensity of Glut-1 was compared to that of EMA in each case of mesothelioma.

5-µm-thick sections were deparaffinised and treated with 3% hydrogen peroxide to block endogenous peroxidase activity, followed by washing in deionised water. Heat induced epitope retrieval with target retrieval solution (Dako) was performed. The slides were stained with primary antibody against GLUT-1 (1:200, polyclonal, Dako). Immunoreactions were detected by the labeled streptavidin-biotin method and visualized with 3, 3-diaminobenzidine, followed by counterstaining with hematoxylin. Red blood cells were used as positive control. The staining results were then divided into negative, focal positive (<30% staining) and diffuse positive (>30% staining). Staining for EMA and desmin for each cases were reviewed and graded negative, focal positive (<30% staining) and diffuse positive (>30% staining). EMA was not performed on 11 of the 17 reactive cases.

Results

The results of immunohistochemistry are summarised in Table 1. Glut-1 expression was demonstrated by membranous staining with or without faint cytoplasmic staining. Positive Glut-1 staining was seen in 14 cases (82.4%) of malignant mesothelioma. One case of biphasic mesothelioma was included. However, the cytology sample of this case contained epithelioid mesothelial cells only. The single sarcomatoid mesothelioma in our series was negative for Glut-1. The two cases of Glut-1 negative epithelioid mesothelioma were strongly positive with EMA. Of the six cases of mesothelioma

showing focal Glut-1 positivity, three were only focally positive for EMA and one was negative for EMA. Only one case of mesothelioma showed focal weak positivity for desmin while the other 16 cases were completely negative. All 17 cases of benign mesothelial proliferation were negative for Glut-1. Of the 17 cases of benign mesothelial proliferation, 11 were strongly positive for desmin, two showed focal positive staining, and four were negative (including the multiloculated benign peritoneal inclusion cyst).

Table 1. Results of Glut-1 staining

	n	Glut-1positive (%)	Negative	Focal	Diffuse
Mesothelioma	17	14 (82.4%)	3	6	8
Epithelioid	15	13 (86.7%)	2	6	7
Biphasic	1	1 (100%)	0	0	1
Sarcomatoid	1	0 (0%)	1	0	0
Benign mesothelium	17	0 (0%)	13		
Peritoneal cyst	1	0 (0%)	1	0	0
Pleural (reactive/ plaque)	6	0 (0%)	0	0	0
Peritoneal fluid	8	0 (0%)	0	0	0
Pericarditis	2	0 (0%)	0	0	0

Discussion

Malignant mesothelioma is a solid tumour of the pleura It is locally aggressive and usually presents with dyspnoea and chest pain (1). Without treatment the prognosis is poor, with median survival ranging from 6 to 18 months (2). In Australia, the malignant mesothelioma-associated mortality has increased since 1965; with 6,129 cases of mesothelioma reported to the Australian mesothelioma register (3). In New Zealand, 554 cases of asbestos related disease were reported to the Asbestos Disease Register between 1992 and 1997. These include 96 cases of mesothelioma, 47 cases of lung carcinoma, 118 cases of asbestosis and 293 cases of pleural abnormalities (4).

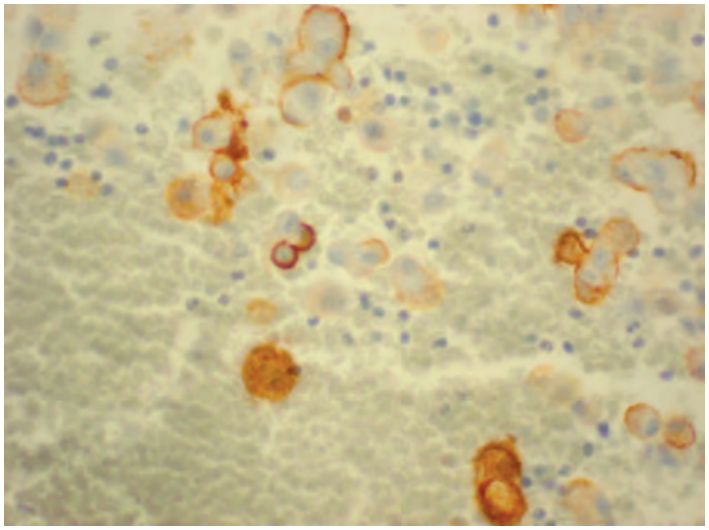
Many risk factors have been identified as contributors to malignant mesothelioma including asbestos, radiation and SV40 virus. Among all, asbestos is the environmental factor that is most frequently associated with mesothelioma. Asbestos belongs to the fibrous silicate group of minerals. These can be structurally divided into two main groups, the serpentine and amphibole groups. The serpentine fibers (mainly chrysotile) are long and flexible. They are mainly used in the textile applications (1). They are soluble and therefore clear faster in the lung. Amphibole fibers (crocidolite, tremolite, anthrophylite and amosite) are mainly used as fire resistant application. They are short and stiff. Their physical structure allows them to penetrate deep into the respiratory tracts where they have a half life of about seven years (very difficult to clear from the lung) (5). Asbestos is thought to induce mutations in mesothelial cells by damaging the mitotic spindle (6,7). It also leads to the formation of reactive oxygen species. Almost 95% of asbestos used internationally is chrysotile. Yarborough concluded in his review that the role of chrysotile fibers in mesothelioma pathogenesis is weak although he suggested the possibility of a threshold for chrysotile (8,9). A discussion of the role of radiation and SV40 poliomavirus in the pathogenesis of mesothelioma is beyond the scope of this article.

The examination of fluid cytology is now part of the daily routine of many pathologists. Better understanding of the morphology of cells in cytologic preparations and the use of various immunohistochemical markers, has enabled distinction between malignant epithelial cells and mesothelial cells in most cases. However, distinguishing reactive mesothelial cells from malignant mesothelial cells in cytology remains challenging. Some say there is limited role of cytology in the primary diagnosis of malignant mesothelioma, but this is debated between cytopathologists and surgical pathologists. The diagnosis of malignant mesothelioma has to be made with certainty, and the International Mesothelioma Interest Group panel recommends that a cytologic suspicion of malignant mesothelioma be followed by tissue confirmation, supported by clinical and radiologic data (10).

Many of the cytologic features of mesothelial cells such as scalloped borders of cell clusters, intercellular windows, biphasic cytoplasm and low nuclear to cytoplasmic (NC) ratio are shared by reactive and malignant mesothelial cells. This makes diagnosis very difficult. Features suspicious for malignant mesothelioma include hypercellular smears with many large clusters of mesothelial cells, usually more than 50 cells in each cluster. The cells in these clusters may be larger than benign mesothelial cells but generally maintain a normal NC ratio (11). The nuclear membranes of malignant mesothelial cells often appear thicker than those of reactive mesothelial cells in cytologic preparations (11). Some authors use macronucleoli and nuclear atypia as criteria for malignancy. However, large nucleoli are often seen in reactive mesothelial cells. Reactive mesothelial cells in the setting of cirrhosis, pulmonary infarct, pancreatitis and uraemia can be highly atypical in appearances (11-13). It is important to remember that there may be a mixture of reactive and malignant mesothelial cells in the same sample. In some cases, especially sarcomatoid mesothelioma, one will expect more reactive mesothelial cells to be exfoliated into the fluid than the malignant cells. In difficult cases, a conservative diagnostic approach should be used. It is often useful to request repeat samples as malignant effusions are likely to reaccumulate quickly and subsequent collections may actually yield a more cellular sample.

Histology is the most reliable way of diagnosing malignant mesothelioma. Mesothelial proliferation that infiltrate into fat, muscle or lung is diagnostic of mesothelioma. While histology is the gold standard, there are several immunohistochemical markers that can assist in the cytological assessment of fluid when mesothelioma is suspected. EMA and desmin are the two most commonly used markers. Most malignant mesothelioma show membranous staining for EMA. While EMA expression is generally expected to be negative in reactive mesothelium, the author has seen cases of histology proven reactive mesothelial proliferation with weak EMA staining. However, in these reactive cases the staining is usually weak and cytoplasmic, unlike the strong and crisp membranous positivity seen in malignant mesothelial cells (Figure 1). Desmin is generally negative in malignant mesothelioma and positive to a certain degree in reactive mesothelial lesions. Desmin positivity in the latter context is cytoplasmic in location. In a study conducted by Attanoos et al, 80% of malignant mesothelioma cases were positive for EMA while only 20% of reactive cases were positive (14). In the same study, 10% of malignant cases were desmin positive while 85% of reactive ones were desmin positive.

Figure 1. Epithelial membrane antigen (EMA) showing strong membranous positivity in the malignant mesothelial cells (cell block).

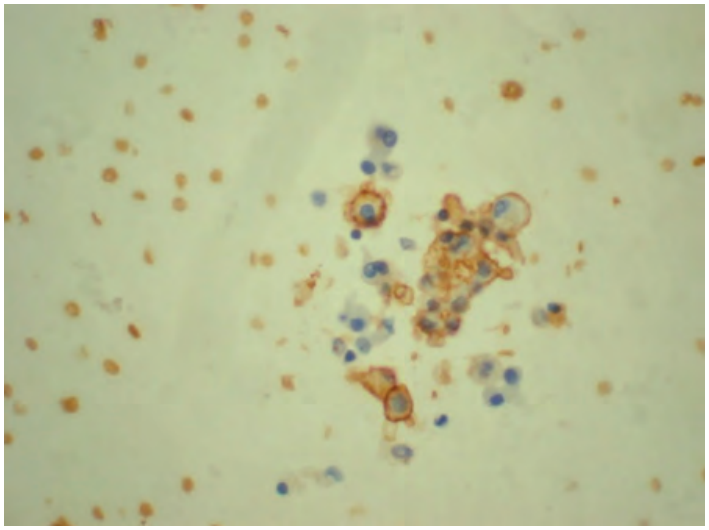


"Glut" stands for glucose transporter, a family of 14 receptors (Glut-

1 to Glut-14) found in mammals and responsible for transporting glucose down a concentration gradient. The human Glut-1 gene has been localized to the short arm of chromosome 1 (1p34.2). Glut-1 is generally not detectable in benign or normal human tissue with the exceptions of erythrocytes, testicular germinal cells, renal tubules, perineurium of peripheral nerve, endothelial cells of blood-brain-barrier and placental trophoblasts (15). In general, malignant cells are found to have higher levels of Glut-1 expression than their benign counterparts. This is hardly surprising considering that malignant cells are expected to consume more energy and therefore require more glucose transporters. High levels of Glut-1 expression have been documented in various malignancies including tumours of the breast, pancreas, cervix, endometrium, lung, mesothelium, colon, bladder, thyroid, bone and soft tissue (16-20). Some authors believe that Glut-1 expression is associated with increased malignant potential and invasiveness of tumours (21-24). Kato et al conducted a study comprised 40 cases of reactive mesothelial proliferation and 48 cases of malignant mesothelioma (15). All 48 cases of malignant mesothelioma were positive for Glut-1 while all the reactive cases were negative.

In our series, Glut-1 positivity was seen in 82.4% of malignant mesothelioma cases (Figure 2). Two epithelioid mesothelioma and one sarcomatoid mesothelioma were negative for Glut-1. Red blood cells were present on all of the slides and were used as internal controls. These showed satisfactory staining. The tendency for sarcomatoid mesothelial cells to stain only weakly for Glut-1 was also documented by Kato et al (15). Glut-1 reactivity in our benign (reactive) group is comparable to that of Kato's study (0% staining with Glut-1). The desmin staining pattern in our study (6% in malignant mesothelioma and 76% in reactive mesothelium) is comparable to that reported by Attanoos et al (14).

Figure 2. Strong membranous staining with Glut-1 in the same cell block



In conclusion, Glut-1 has proven itself as a useful adjunct to routine fluid cytology in the investigation of patients with possible malignant mesothelioma. However, it is important to recognize the fact that reactive mesothelial cells are often exfoliated at the same time as neoplastic cells, contributing to a variable staining pattern within the same sample. Negative staining with Glut-1 does not rule out the possibility of malignant mesothelioma, especially when clinical suspicion is high. Another limitation of the use of Glut-1 in cytology is the frequent heavy background blood staining of the samples, making assessment difficult. Glut-1 is best interpreted in conjunction with other markers such as EMA and desmin. At the end of the day, clinical and radiological correlation is crucial.

Acknowledgements
Thank you to Dr Margaret Lovell-Smith and Dr Chris Van Vliet, pathologists at Labplus for their support in this study. Also thank you to technical staff at Labplus for their assistance in tissue processing.

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In regard to the macrocytosis in COPD paper by Wu and colleagues, the Editor of the Journal, Rob Siebers, is a co-author. In order of transparency and avoidance of conflict of interest the handling of this article was independently done by the Deputy Editor who oversaw the peer review process and made the final editorial decision of acceptance. The Journal's Editor had no input in the editorial process and was treated the same as any other author submitting to the Journal.

Each year the NZIMLS Council invites a member, who has made a significant contribution to medical laboratory science, to deliver the TH Pullar Memorial Address at the Annual Scientific Meeting. This prestigious address is in honour of Dr TH Pullar, a pathologist who was a champion and great friend of New Zealand medical laboratory scientists. Thos Pullar was involved in their training and welfare, drafting conditions of employment and preparing syllabi and examinations for their professional training. This year Christine Pry, from Abbott Diagnostics, was honoured to deliver the TH Pullar Memorial Address. Her address, entitled "Believe in yourself, and anything is possible" is in this issue.

In this issue is another Journal questionnaire. You may have noticed that over time the Journal questionnaire has required more in-depth or multiple answers for the questions. Despite this, some members write very sparse answers. For instance, question one from the August 2010 questionnaire asked members to name the three types of von Willebrand disease. Quite a few just simply answered "Type 1, Type 2 and Type 3". The fuller and correct response should have been "Type 1 is a partial quantitative deficiency in vWF, Type 2 is a qualitative defect and Type 3 is an extreme quantitative defect (where vWF is absent). Similarly, question 6 asked for the principles of measurement of the vWF:RCo and HaemosIL methods. Just answering "by turbidometry" meant that those members only got a fraction of the mark for that question as they did for the above simplistic answer to question one. Thus some members, because they also missed out answers on other questions, did not achieve the minimum of eight (full) correct answers and were not awarded their 5 CPD points. Please read the questions and articles carefully before answering the questions in detail.

Prevalence of extended spectrum ß-lactamase among Gram-negative bacteria isolated from surgical wound and blood stream infections in Benin City, Nigeria

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Abstract

Objectives: To determining the prevalence of extended spectrum β-lactamase producing Gram negative bacteria (ESBL) from surgical wound and blood stream infections in a teaching hospital where third generation cephalosporins are used as blind therapy.

Methods: The presence of ESBL was determined among 806 Gram negative bacilli from surgical wound (765) and blood infections (41) using double disc synergy method. Disc susceptibility test was also performed on all isolates using standard techniques.

Results: Gram negative bacteria isolated from blood (14.6%) had a significantly higher prevalence of ESBL producers compared with isolates from surgical wounds (14.6% vs 2.1% respectively; OR: 8.0, 95% CI: 3.0-21.8, P < 0.0001). Bacterial isolates that produced ESBL were more resistant to the antibacterials used compared with non-ESBL producers.

Conclusions: An overall prevalence of 2.7% of ESBL producing Gram-negative bacteria was observed and isolates from blood were 3 to 22-fold more likely to produce ESBL. Prudent use of antibiotics is advocated.

Key words: β-lactamase, Gram-negative bacteria, prevalence, infections, third generation cephalosporins

N Z J Med Lab Sci 2010; 64: 74-76

Introduction

The incidence of extended spectrum β-lactamase [ESBL] producing strains among clinical isolates has been steadily increasing over the past years resulting in limitation of therapeutic options (1). Most ESBL are mutant of classical TEM-1, TEM-2 and SHV-3 β-lactamase types and, unlike these parents enzymes, ESBL hydrolyses oxyimino-aminothiazolyl cephalosporins such as cefuroxime, cefotaxime, ceftriaxone, ceftizoxime, ceftazidime cefpirome and cefeprine as well as penicillins and other cephalosporins except cephamycins (2). ESBL-mediated resistance to cephalosporins is not always obvious in disc or dilution test. However, such ESBL have been associated with clinical failure in patients and in experimental animals (2-4).

Antimicrobial usage in life threatening conditions without laboratory guidance is sometimes warranted. In life threatening conditions such as blood stream infections and antibiotics coverage during surgery, such antibiotic usage is guided by the selective toxicity and broad spectrum of the antimicrobial agents as well as susceptibility pattern of the given area. β-lactamase antibiotics, especially cephalosporins, are the drug of choice in our locality. The third generation cephalosporins used are taken parenterally and are expensive, thus public abuse of these agents is low. These drugs have been shown to be very effective both in susceptibility tests and cure of infections in our setting. Our hospital antibiotic policy is partly based on the use these agents in life threatening conditions, such as blood stream infections before laboratory results become available. These agents are also used during surgery to prevent infections as they are broad spectrum in action. However, we have observed an increase in microbial isolates associated with surgical wound breakdown and blood infections.

ESBL producing bacterial strains have been reported worldwide and from various clinical specimens (5) but there is no report on ESBL prevalence in our locality. Against this background this study aimed to determine the prevalence of ESBL among Gram negative bacteria causing blood infections and surgical wound infections as well as their susceptibility patterns.

Materials and methods

Bacterial isolates

A total of 806 consecutive non-repetitive bacterial isolates from surgical wounds (765) and blood stream infections (41) were used for this study. The isolates included *Escherichia coli, Klebsiella* species, *Enterobacter* species, *Citrobacter* species, *Proteus* species, *Providencia* species, *Acinetrobacter* species, *Alcaligenes* species and *Pseudomonas aeruginosa*. All isolates were obtained from the Medical Microbiology Laboratory of the University of Benin Teaching Hospital (a tertiary hospital with a referral status) in Benin City, Nigeria and were identified using standard techniques.

ESBL detection

ESBL was detected using the method described by Livermore and Brown (2). Briefly, test organisms were emulsified in sterile water and the turbidity matched with 0.5 McFarland standard. Once

matched, a sterile cotton-wool swab was dipped in the organism suspension and excess liquid was removed by turning the swab on the side of the test tube. The entire surface of Mueller-Hinton agar plate (Scharlau, Spain) was seeded by swabbing in three directions with the swab. A disc containing 30μg amoxicillin–clavulanate (Oxoid, England) was placed at the centre of the agar plate close to a 30μg ceftazidime disc (30mm apart). The plates were incubated overnight at 37^{0}C and ESBL production was inferred as positive if there was an expansion of the zone of inhibition between the ceftazidime and the amoxicillin–clavulanate disc.

Susceptibility testing
Disc susceptibility tests were performed on all bacteria isolates using the BSAC method (6).

Results
A total of 22 (2.7%) out of 806 bacterial isolates were positive for ESBL. The isolates from blood stream infections were more likely to produce ESBL than isolates from surgical wound infections as shown in Table 1 (blood vs wound: 14.6% vs 2.1%; OR: 8.0, 95% CI: 3.0-21.8, P < 0.0001). The bacteria that produced ESBL showed variable susceptibility profile (Table 2). While the genera *Enterobacter, Proteus, Providencia and Alcaligenes* that produce ESBL were resistant to all the antibacterial agents used, the susceptibility of other genera ranged from 25% to 100%, depending on the antibacterial agent. The susceptibility of non-ESBL producing isolates showed moderate to high susceptibility to all antibacterial agents tested (Table 3).

Table 1. Prevalence of ESBL production among isolates from different sources

Organisms	No of EBSL producers	Surgical wound	n	Blood (%)	n	Total
Escherichia coli	126	7 (5.6%)	2	2 (100%)	128	9 (7.0%)
Klebsiella species	117	3 (2.6%)	19	1 (5.3%)	136	4 (2.9%)
Enterobacter species	11	2 (18.2%)	8	0	19	2(10.5%)
Citrobacter species	21	0	0	0	21	0
Proteus species	127	2 (1.6%)	4	0	131	2 (1.5%)
Providencia species	62	0	5	1 (20.0%)	67	1 (1.5%)
Acinetobacter species	51	0	2	2 (100%)	53	2 (3.8%)
Alcaligenes species	63	0	0	0	63	0
Pseudomonas aeruginosa	187	2 (1.1%)	1	0	188	2 (1.1%)
Total	765	16 (2.1%)	41	6 (14.6%)	806	22(2.7%)

Table 2. Susceptibility profiles of ESBL producers

Organisms	Antibacterial agents (μg/disc)				
	OFX(5)	CN (10)	CAZ (30)	AUG (30)	CXM (30)
Escherichia coli (n = 9)	4 (44.4%)	0	0	4 (44.4%)	0
Klebsiella species (n = 4)	3 (75.0%)	0	0	1 (25.0%)	0
Enterobacter species (n = 2)	0	0	0	0	0
Proteus species (n = 2)	0	0	0	1 (50.0%)	0
Providencia species (n = 1)	0	0	0	0	0
Acinetobacter species (n = 2)	0	0	0	0	0
Pseudomonas aeruginosa (n = 2)	1 (50.0%)	1 (50.0%)	2 (100%)	0	0

OFX = ofloxacin, CN = gentamicin, CAZ = ceftazidime, AUG= amoxicillin-clavulanate, CXM = cefuroxime.

Table 3. Susceptibility profiles of non-ESBL producing isolates

Organisms	Antibacterial agents (μg/disc)				
	OFX(5)	CN (10)	CAZ (30)	AUG (30)	CXM (30)
Escherichia coli (n = 119)	119 (100%)	94 (79.0%)	83 (69.8%)	101 (84.9%)	86 (72.3%)
Klebsiella species (n = 132)	105 (79.6%)	85 (64.4%)	98 (74.2%)	111 (84.1%)	87 (65.9%)
Enterobacter species (n = 17)	14 (82.4%)	6 (35.3%)	7 (41.2%)	9 (52.9%)	6 (35.3%)
Citrobacter species (n = 21)	18 (85.7%)	11 (52.4%)	12 (57.1%)	17 (81.0%)	9 (42.9%)
Proteus species n = 129)	102 (79.1%)	116 (89.9%)	100 (77.5%)	119 (92.3%)	116 (89.9%)
Providencia species (n = 66)	58 (87.9%)	51 (72.3%)	53 (80.3%)	62 (93.9%)	43 (65.2%)
Acinetobacter species (n = 51)	44 (86.3%)	40 (78.44%)	34 (66.7%)	47 (99.2%)	41 (80.4%)
Alcaligenes species (n = 63)	56 (88.9%)	48 (76.2%)	52 (82.5%)	56 (88.9%)	57 (90.5%)
Pseudomonas aeruginosa (n = 186)	144 (77.4%)	93 (50.0%)	106 (57.0%)	152 (81.7%)	161 (86.6%)

OFX = ofloxacin, CN = gentamicin, CAZ = ceftazidime, AUG= amoxicillin-clavulanate, CXM = cefuroxime

Discussion
The incidence of ESBL producing strains among clinical isolates has resulted in the limitation of therapeutic option (1) and the widespread use of third generation cephalosporins and aztreonams is believed to be the major cause of mutation that has led to the emergence of ESBL (7). Isolates that produces ESBL are common causes of sepsis, pneumonia, urinary tract infection and post surgical infection in patients in acute care hospitals (8). Lack of data on its prevalence in our society necessitated this study which aimed to determine the prevalence of ESBL among surgical wound and blood stream infections as well as susceptibility profiles of ESBL producing and non-producing Gram negative bacteria.

A total of 22 (2.7%) out of the 806 Gram-negative bacteria were positive for ESBL production and isolates from blood cultures were more likely to produce ESBL compared to isolates from surgical wounds. The finding that isolates from blood culture had a higher prevalence of ESBL production compared with isolates from surgical wound agrees with a previous study (9). Previous exposure to third generation cephalosporins has been reported to be associated with infection caused by ESBL (10). Third generation cephalosporins such as ceftriaxone, ceftazidime and cefotaxime are used as blind therapy in our institution, especially in suspected cases of septicemia. This may explain the finding of our study.

Although most studies have showed *Escherichia coli* and *Klebsiella* spp as the most prevalent ESBL producing Gram negative bacteria (11), *Enterobacter* spp was the most prevalent ESBL producing Gram-negative bacteria from surgical wounds while *Escherichia coli* and *Acinetobacter* were the most prevalent ESBL producing bacteria from blood stream infections in our study. The reasons

for these differences are unclear. Ofloxacin, gentamicin and amoxicillin–clavulanate were the antibacterials active against ESBL producing Gram negative bacteria with 25 – 100% of isolates susceptible. Although amoxicillin–clavulanate showed in–vitro activities against ESBL isolates, due to the presence of clavulanic acid ESBL enzymes have been reported to confer resistance to all penicillins and all cephalosporins, including the sulbactam and clavulanic acid combinations (11). Therefore, the in–vitro activity of amoxicillin–clavulanate does not translate to in–vivo activity. It has been reported that ESBL-mediated resistance is not obvious in disc or dilution susceptibility testing but is associated with clinical failure (2-4). This may explain the results of amoxicillin – clavulanate in our study.

Susceptibility of non-ESBL producing isolates revealed a moderate to high susceptibility to the antibacterial agents used in our study. This finding indicates that ESBL producing isolates are usually more resistant to antibacterial agents compared to non-ESBL producing isolates. This results in limitation to therapeutic options (1).

A limitation of our study was that speciation of *Enterobacteriaceae* was not possible as the number positive for ESBL were too small and speciation would have rendered the susceptibility results difficult to discuss.

In conclusion, our study revealed a prevalence of 2.7% of ESBL producing Gram-negative bacteria isolated from surgical wounds and blood stream infections. Isolates from blood stream infections had a 3 to 22 fold increasing likelihood of producing ESBL. EBSL producing isolates are more resistant to antibacterial agents and prudent use of antibacterial agents is advocated to stem the tide.

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Author contributions: Richard Omoregie took part in the study design, generated and analysed the data and substantively drafted the article. Isaac Igbarumah took part in the study design, generated the data and substantively drafted the article. Christopher Egbe took part in the study design, generated the data and substantively drafted the article. Helen Ogefere took part in the study design, analysed the data and substantively drafted the article. Philomena Ogbogu took part in the study design, generated the data and substantively drafted the article. The authors have no conflicts of interest to declare.

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HLA-B27 polymorphism associated with disease in a New Zealand population

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Abstract

Objectives: To characterise the subtypes of HLA-B27 in disease associated patients in the North Island of New Zealand.

Methods: The subjects were 194 patients from the North Island of New Zealand. These had previously been positively tissue-typed for HLA-B27 owing to its associations with spondylarthropathies and anterior uveitis. The subjects gave informed consent to further testing of their samples, which were HLA-B27 subtyped using DNA sequence based typing. Statistical analysis of the data was performed using the chi square test with Yates' correction and Pearson's chi square test.

Results: Nine different subtypes were described in this study. HLA-B*2701, B*2702, B*2704, B*2705, B*2706, B*2707, B*2709, B*2710 and B*2715. Within the study the prevalence of B*2704 was significant in Asians ($p<0.001$) and the prevalence of B*2705 was significant in Europeans ($p<0.001$). Of the three major subtypes identified (B*2702, B*2704 and B*2705), none showed a statistical association to disease category. HLA-B27 homozygosity was not significantly different to the healthy population ($p=0.918$).

Conclusions: HLA-B27 subtypes were analysed in disease associated patients of the New Zealand North Island population. The subtypes found reflected the HLA-B27 subtypes found in previous studies of the different ethnicities involved.

Key words: HLA-B27, subtypes, disease association, spondylarthropathies, uveitis.

N Z J Med Lab Sci 2010; 64: 78-84

Introduction

The class I human leukocyte antigen (HLA) system is part of the adaptive immune response and is involved in presentation of peptides to cytotoxic T lymphocytes (CTL)(1). HLA antigens are encoded for by the major histocompatibility complex (MHC) region of genes on the short arm of chromosome 6 (1). HLA-B27 is a serological specificity that represents a family of 53 allelic subtypes to date (HLA-B*2701 to B*2754, excluding B*2722) (2). The spondylarthropathies (SpA) comprise a family of chronic inflammatory diseases affecting the spine, joints, eyes and skin. HLA-B27 is strongly associated with ankylosing spondylitis (AS), related SpA and anterior uveitis (AU) (3-5). The New Zealand Tissue Typing Laboratory currently ascertains the presence or absence of HLA-B27 in referred samples by DNA polymerase chain reaction (PCR) with sequence specific primers (SSP) technique, using the AllSet+™ Gold SSP HLA-B27 Low Res Kit (Invitrogen™ Carlsbad, Ca, USA). This technique does not specify the allelic subtype of HLA-B27 in any given sample. Epidemiological studies have implicated B*2701 to B*2710, B* 2713, B*2714, B*2715 and B*2724 with AS (6-26). In certain populations however, B*2706, B*2707, B*2708 and B*2709 appear to have a protective role (14,16,19,21,24,26-29). B*2705, and B*2704 to a lesser degree, have been shown to be associated with AU (30,31). There is little information on disease association with the other HLA-B27 subtypes.

The advent of DNA typing to the sequence level and the discovery that some HLA-B27 subtypes confer protection against SpA (14,16,19,21,24,26-29) provides the rationale for research in this area. An understanding of HLA-B27 subtypes positively and negatively associated with SpA may give insights into the pathogenesis of these disorders and could pave the way to future treatments. The objective of this study was to characterise the HLA-B27 subtypes in patients from the NZ North Island population whose samples have been referred for HLA-B27 testing due to clinical symptoms characteristic of SpA and uveitis.

Materials and methods

Subjects

Between April 2006 and April 2007 the NZ tissue typing laboratory tested 1894 patients from the North Island of NZ for HLA-B27 due to symptoms of associated disease. This tissue type was demonstrated in 338 patients. Of these, 194 gave informed consent for their sample to be further subtyped and were included in the study. Participants in the study declared their own ethnic group. Clinical symptoms were taken from the request forms of the original samples and classified as SpA, uveitis or no clinical information available. The study received multi-region ethical approval through the Health Research Council of NZ (application: MEC/07/11/146).

DNA sequencing

DNA sequence based typing (SBT) was performed based on the method described by Dunn et al (32). Briefly: a heterozygous amplicon of the entire HLA-B locus was produced from each sample following manufacturer's instructions for the Expand High Fidelity PCR System (Roche, Basel, Switzerland) and using primers for the 5' and 3' untranslated regions of the gene (Table 1). The PCR product was compared to DNA Molecular Weight Marker VII (0.081-8.57 kbp) (Roche, Basel, Switzerland) by agarose gel electrophoresis on a 0.8%-1.2% agarose gel electrophoresed for 1 hour at 85v. PCR product purification was performed using Agencourt® AMPure® beads (Beckman Coulter Fullerton, CA, USA), following manufacturer's instructions. The purified PCR product concentration and purity were checked on a NanoDrop® ND-100 spectrophotometer (NanoDrop Technologies, Inc. Wilmington, DE, USA). Big Dye chemistry was used to perform cycle sequencing using ABI Prism® BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, Ca, USA). Exons 2 and 3 of amplified HLA-B were sequenced in both directions using 4 different HLA-B specific primers (Table 1). Agencourt® CleanSEQ® beads (Beckman Coulter Fullerton, CA, USA) were used, following manufacturer's instructions, to remove unincorporated dye terminators. DNA sequenced fragments were separated and analysed by capillary electrophoresis on a Hitachi 3130x/ Genetic Analyser (Applied Biosystems, Foster City, Ca, USA).

Sequence analysis

DNA sequences were compiled and analysed against a database of HLA alleles using SBTengine software version 2.8.0.0 (Genome Diagnostics B.V., Utrecht, The Netherlands) (see Figure 1). The

software assigned allele type was recorded.

Statistical analysis
The differences in allele frequencies in the different population groups were analysed using the chi-square test (Yates' correction). This test was also used to analyse the association of the disease groups with the different subtypes. Pearson's chi-square test was used to calculate the significance of B*27 homozygosity in this study.

A population of 1,703 blood donors, who had been tissue typed, was used to calculate the gene frequency of HLA-B*27 (the proportion of HLA-B*27 alleles out of all the HLA-B genes in the population (33)) and phenotype frequency of HLA-B27 (the proportion of the population with the HLA-B*27 allele, regardless of whether it occurs in a heterozygous or homozygous state (33) in the healthy population.

Results

Out of the 194 patients tested, 158 (81.44%) were European, 16 (8.24%) were Asian, 12 (6.19%) were Māori or European/Māori. The remaining eight (4.12%) were from other population groups.

Ninety six (49.48%) subjects were male and 98 (50.52%) were female. Eleven (11) males and 12 females had symptoms of uveitis. Fifty seven (57) males and 59 females had symptoms of SpA. There was no significant difference between males and females with symptoms of disease.

One hundred and sixteen (116) patients had symptoms of SpA on their sample request forms and 23 had symptoms of uveitis. Two of these patients had symptoms of both SpA and uveitis. No clinical information was available for 57 patients.The distribution of the disease categories across the different ethnic groups is represented in Table 2.

A total of 204 HLA-B*27 subtypes were identified in this study, One hundred and eighty four (184) heterozygous patients each had one HLA-B*27 subtype. Four patients were heterozygous for HLA-B27 subtypes: two had B*2702, B*2705, one had B*2701, B*2705, and one had B*2704, B*2706. Five patients were homozygous for the B*2705 subtype and one patient was homozygous for the B*2704 subtype. Homozygosity of HLA-B27 in disease associated patients was not significantly different to the general population (p=0.918).

Of the 204 total, nine different subtypes were identified: B*2701, B*2702, B*2704, B*2705, B*2706, B*2707, B*2709, B*2710 and B*2715. B*2705 was the most common subtype occurring 172 times, next was B*2704 occurring 15 times; B*2702 occurred ten times, B*2701 occurred twice and B*2706, B*2707, B*2709, B*2710 and B*2715 occurred once each. The distribution of HLA-B*27 alleles across the different population groups is represented in Table 3. The association of the prevalence of the major alleles, B*2702, B*2704 and B*2705, were compared for each population group. Of significance were B*2705 in Europeans ($p<0.001$) and B*2704 in Asians ($p<0.001$).

The significance of B*2702, B*2704 and B*2705 to each disease category was calculated for those patients for whom clinical information was available. There was no significance of any of these alleles to any disease category.

A new variant of B*2704 was discovered during this project. A nucleotide change in Exon 3 at position 435 (AAG-AAA) resulted in a silent mutation in a lysine residue.

The HLA-B*27 gene frequency in the healthy population was calculated as 0.046. The HLA-B27 phenotype frequency was calculated as 0.089.

Table 1. Amplification and sequencing primers used

Amplification Primers				
Primer	**Direction**	**Location**		**Sequence (5'→3')**
HLA5BUT	forward	-320 to ATG start		ggCAgACAgTgTgACAAAgAggC
3B38	reverse	+175 after term		CTggggAggAAACACAggTCAgCATgggAAC
Sequencing Primers				
Primer	**Exon**	**Direction**	**location**	**Sequence (5'→3')**
5BIn1-121	Exon 2	forward	Intron 1, 121-140	ggACCgCAggCgggggCKCA
3B44	Exon 2	reverse	Intron 2, 496-514	ggATgggAgTCgTgACCT
5BIn2	Exon 3	forward	Intron 2, 226-243	ACKgKgCTgACCgCggg
3BIn3-1027	Exon 3	reverse	Intron 3, 1027-47	ggCCATCCCCggCgACCTATA

Table 2. Disease categories and ethnicity

Disease	**Population Group**				**Total***
	European	Asian	Māori, Euro/Māori	Other	
SpA	96	9	7	4	116
uveitis	15	4	3	1	23
unknown	47	4	3	3	57

* Two patients had symptoms of both SpA and uveitis, thus the total occurrences of disease categories exceeds the total number of patients in the study.

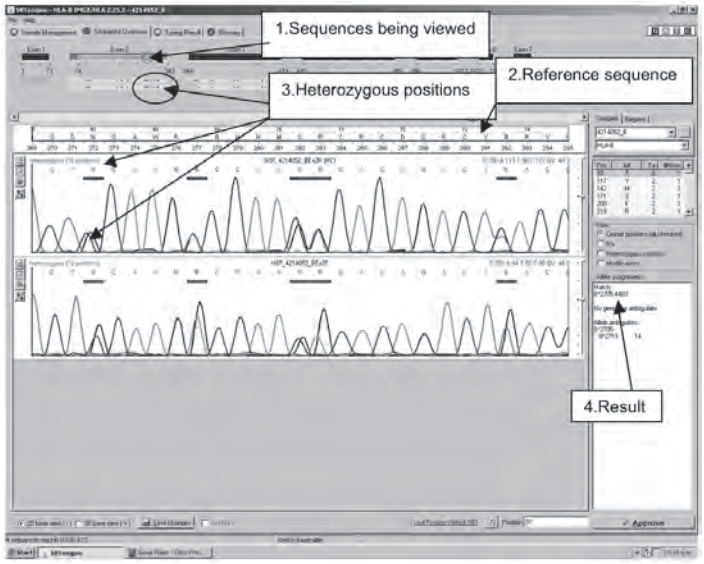
Table 3. HLA-B*27 allele frequencies across the entire group.

Allele	**Population Group**				**Total***
	European	Asian	Māori, Euro/Māori	Other	
B*2701	2	0	0	0	2
B*2702	10	0	0	0	10
B*2704	0	12	1	2	15
B*2705	152	4	11	5	172
B*2706	0	1	0	0	1
B*2707	0	0	0	1	1

B*2709	1	0	0	0	1
B*2710	1	0	0	0	1
B*2715	0	1	0	0	1

*The total number of HLA-B*27 alleles exceeds the number of patients in the study because some patients were homozygous for HLA-B*27.

Figure 1. SBT engine software sequencing of exons 2 and 3 of the HLA-B gene. Individual nucleotide bases are indicated by coloured peaks. **1.** The sequence being viewed is indicated by a cursor at the top of the screen. **2.** The sequence obtained is compared against the reference sequence for HLA-B. **3.** Heterozygous positions are indicated by an asterisk in the directional arrows at the top of the screen and by two superimposed peaks below the International Union of Biochemistry code. **4.** The software assigned type is given at the right of the screen.



Discussion

The mechanisms of HLA-B27 association with SpA

The strong association of HLA-B27 to SpA and uveitis has been known since 1973 (3,4). The mechanisms of the association between HLA-B27 and the SpA remain uncertain. The current hypotheses have been reviewed and suggest three main lines of thought (34-38):

1. Molecular mimicry may occur through HLA-B27 presenting peptide epitope from gram negative bacteria such as *Klebsiella* to CTL. Some of the CTL may cross-react with self-ligand, eliciting an autoimmune inflammatory response (39).
2. Before being expressed on the cell surface, the HLA-B27 heavy chain proteins fold slowly and they can misfold and accumulate in the endoplasmic reticulum (ER). The resultant ER stress may activate unfolded protein and subsequently activate nuclear factor-κB, inducing pro-inflammatory cytokines (40,41).
3. HLA-B27 heavy chain proteins can form covalent homodimers which are expressed on the cell surface. Leukocyte receptors recognise these with a specificity pattern that is distinct from, yet overlaps that of the recognition of normal HLA-B27 heterodimers. The interaction with homodimers may alter normal immunomodulatory mechanisms and potentially cause excess pro-inflammatory cytokine release (42).

Other genetic factors and SpA

An assessment of the genetic risk for AS has identified that genes linked to the MHC contribute only 31% of the total genetic risk for AS (43). Other HLA types shown to have an association with AS are HLA-B60, HLA-DRB1 (44,45) and HLA-B*1403 (46). Research has identified some killer cell immunoglobulin like receptors also have an association with AS in conjunction with HLA-B27 (47). A 270kb region of the MHC class III region of chromosome 6, containing 23 genes outside of the HLA-B27 region has been identified as conferring susceptibility to AS (45). These considerations need to be taken into account when considering HLA-B27 subtypes' association to SpA.

Uveitis

Uveitis is a common complication of AS in some patients (5,30,31,48). HLA-B27 associated uveitis however, occurs regardless of AS and is considered a separate clinical entity (31). A recent study of HLA-B27 in uveitis in a NZ population has identified 36% of bilateral uveitis patients and 47% of unilateral uveitis patients as being HLA-B27 positive (48). Of these patients, 41% had AS. In the present study two of patients with uveitis symptoms also had SpA symptoms; however, the numbers were too small to draw reliable conclusions.

HLA-B27 typing techniques

HLA-B27 has been typed using various techniques (49). Initial studies used microcytotoxicity methods to serologically determine the presence or absence of HLA-B27 on the surface of lymphocytes (3). Flow cytometry techniques make use of monospecific antisera and fluorescent labelling to determine HLA-B27 positivity (12,50). PCR-based DNA technology for tissue typing has allowed the presence of HLA-B*27 to be determined at the genetic level. PCR-SSP technique uses DNA primers to directly amplify nucleotide sequences of interest, which can then be detected by gel electrophoresis (49). Low to high resolution DNA typing is possible with this technique. A PCR melting assay has also been described (50). Sequence specific oligonucleotide (SSO) technique involves all allelic sequences from the locus of interest being amplified by PCR. PCR products are then hybridised to oligonucleotide probes specific to the DNA sequence of interest. Biological labelling allows the sequences of interest to be distinguished. This technique can be used for low or high resolution HLA typing (49). DNA sequencing gives high resolution typing with few ambiguities (32,49,51). Different groups have described SBT techniques for HLA typing as well as ways to reduce ambiguities (32,51). A limitation of the sequencing done in this project was the occurrence of ambiguities. In this circumstance the most likely combination of alleles was chosen. Further sequencing with more specific primers or of regions outside the exon 2 and 3 regions could have helped resolve this (52).

HLA-B27 subtype and epidemiology

According to 2006 census data, the ethnic groups of the NZ North Island population are 72.31% European or other ethnicity (including New Zealander), 17.08% Māori, 11.25% Asian, 8.86% Pacific Peoples, and 1.03% Middle Eastern/Latin American/African (53). This compares with 81.44% European, 8.24% Asian, 6.19% Māori or European/Māori and 4.12% from other population groups in this study. The apparent overrepresentation of Europeans and underrepresentation of other ethnicities in this study is intriguing. Participants in this study do not, however, represent a random selection of the NZ North Island population. Sources of bias for the representation of population groups here may include differences in bringing symptoms to the attention of a doctor and different levels of response to the invitation to participate in the study. There were similar numbers of males and females in this study: 49.48% of subjects were male and 50.52% of subjects were female. This compares favourably with the 48.88% males and 51.12% females in the NZ North Island population in 2006 (53). The ratio of males to females was similar in the SpA and uveitis disease groups and amongst the different ethnicities.

Europeans

The largest population group represented in this study was European (81.44%), and B*2705 was the most common subtype found. The association of B*2705 with Europeans was significant ($p<0.001$). B*2705 is the predominant subtype amongst Europeans (8,16,17,26,42). It is thought that the other HLA-B27 subtypes have

evolved from B*2705 (54,55). B*2701 and B*2702 were found exclusively in Europeans in this study; however, the numbers of these subtypes were not high enough to be significant. Previous research has noted B*2702 as a major HLA-B27 subtype in European populations (8,16,17,26,41). B*2702 is thought to have entered the European population from the Middle East (16). B*2702 is the most common subtype in Ashkenazi Jews with AS in Israel (7) and shows a high incidence in Turkish populations (10,22).

Asians

Asians comprised 8.24% of the study. The frequency of HLA-B27 subtypes has been studied in various populations in Asia. Studies of the Han Chinese (12,20), Taiwanese (12,28,56), Malays (57), Indonesians (21) and research of the Asian population have found B*2704 to be the most common subtype (6,19). This allele has also been described in ethnic groups from Western India at various frequencies (25,58). Indeed, B*2704 is thought to be almost completely restricted to Asians and their descendants (6). This was reflected in the present study where B*2704 was the most common subtype in the Asian ethnic group and the association was significant ($p<0.001$). Interestingly, and in contrast to other Asian populations, the most common HLA-B27 subtype in Korean patients with AS is B*2705 (18).

Māori

B*2704 and B*2705 were the only subtypes described in Māori. There has been little previous research on HLA-B27 subtypes in this population group. One worldwide study of HLA-B27 subtypes involving 12 samples of Māori also found only B*2704 and B*2705 subtypes (16). The small numbers of Māori and Māori/Europeans in this study prevent reliable conclusions being drawn from the data for this group. The prevalence of HLA-B27, AU and SpA in Māori are unknown and could be an additional source of bias that influenced the representation of Māori in this study. A recent study of human genetic diversity in the Pacific estimated European admixture in Māori to be approximately 12% (59). Admixture between Europeans and Māori may have altered the prevalence of HLA-B27, its subtypes and associated disease in the Māori population.

HLA-B27 subtypes of interest

B*2706

Some HLA-B27 subtypes have been shown to confer protection against SpA in different populations. B*2706 has been previously shown to have a negative association to AS in Thais (16,19), Singapore Chinese (27) and Indonesians (21) and was absent in a Taiwanese population of AS patients (28). All participants in the present study were originally HLA-B27 typed to aid diagnosis of HLA-B27 associated disease. B*2706 was found in one patient in this study who was heterozygous for HLA-B*27, also having a B*2704 allele. This patient was Filipino and had symptoms of SpA. B*2704, B*2706 heterozygosity has been previously described in AS patients in Indonesia (16,60). One study of two mixed Chinese/Indonesian families suggested that the existence of B*2704, B*2706 heterozygotes with SpA symptoms is evidence that although B*2706 is not associated with SpA, it does not confer protection either (60). Two patients with AS in China have been identified as carrying B*2706 (16). Examinations of the low association of B*2706 with AS compare it with B*2704, the common disease associated subtype in the same populations and its most closely related allele (61,62). B*2704 and B*2706 differ in the β-pleated sheets of the peptide binding groove of the molecule. An amino acid difference at position 114 results in histidine (His) and aspartic acid (Asp) respectively in pocket D and a difference in position 116 results in Asp and tyrosine (Tyr) respectively in pocket F (2,61,62). It has been proposed that differences in peptide binding between B*2704 and B*2706 is responsible for their differential association with AS(27,61,62).

B*2709

B*2709 was demonstrated in one patient in the study of European origin. Unfortunately, no clinical details were available for this patient. B*2709 is thought to have a protective role against AS in Sardinians (16,24,29); however, this subtype has been previously described in an Italian patient with AS (23). Furthermore, B*2705 and B*2709 are on distinct HLA haplotypes in the Sardinian population which allows for involvement of other genetic factors on the HLA haplotype in the protection from, or susceptibility to AS (63,64). The differences between B*2709 and the common disease associated subtype B*2705 have been studied (65-68). These two alleles differ by one amino acid at position 116 resulting in His and Asp respectively in the peptide C-terminus accommodating F pocket (2,67). Differences between these subtypes do not result in a difference in the accumulation of heavy chain proteins in the ER (67). B*2705 is more flexible than B*2709 in the peptide groove and the C-terminal peptide anchor can become partially detached. These structural differences may influence HLA-B27 interactions with CTL (66).

B*2707

Our study found B*2707 in one patient who was Lebanese. Unfortunately, no clinical details were available on this patient. B*2707 has been described as having a possible protective role against AS in the Greek Cypriot population (26). A study of an Israeli population (7) and another of the Han Chinese (20) found B*2707 only in healthy controls; however, only two and three respective examples of this allele were identified. Several other studies have, however, demonstrated this allele in patients with AS (9,16,17,19,21,22,24). Interestingly, B*2707 lacks Asp116 as do B*2706 and B*2709 (69). The association of B*2707 with AS is not explained by the misfolding of heavy chains (70).

B*2715

B*2715 has been previously described in a two Thai patients with AS (6). This disease association was confirmed in the present study where B*2715 was described in an Asian patient with SpA symptoms.

B27 homozygosity

Five European subjects in this study were homozygous for B*2705 and one Asian patient was homozygous for B*2704. The levels of HLA-B27 homozygosity were not significantly different to the general population ($p=0.918$). HLA-B27 homozygosity has been shown to influence the severity of AS (71). Whether HLA-B27 homozygosity confers an increased risk of AS or not is contested (71,72). AS patients that are HLA-B27 homozygotes are also more likely to develop other SpA and uveitis (71,73). The B*2704 homozygote identified in this study had symptoms of uveitis.

Further work in this field could include epidemiological studies of HLA-B27 subtypes in a healthy NZ population. A comparison of HLA-B27 subtypes in between the disease associated and healthy populations could be used to calculate the relative risk of disease for different ethnic groups in NZ. A limitation of this study was the lack of clinical symptoms available on historic sample request forms. Seeking more complete clinical information from physicians would be helpful in future studies.

In conclusion, this project analysed HLA-B27 subtypes in 194 disease associated patients in the North Island of NZ. Nine subtypes were described: B*2701, B*2702, B*2704, B*2705, B*2706, B*2707, B*2709, B*2710 and B*2715. Within the study B*2704 was significant to the Asian ethnic group ($p<0.001$) and B*2705 was significant to the European ethnic group ($p<0.001$). The three major subtypes identified were B*2702, B*2704 and B*2705. None of these showed a statistical association to disease category. HLA-B27 homozygosity was not significantly different to the healthy population ($p=0.918$). The subtypes found reflected the HLA-B27 subtypes found in previous studies of the different ethnicities involved.

Acknowledgements

The following are gratefully acknowledged: Dr. Paul Dunn for guidance and supervision; the support of the NZ Blood Service and the staff of the NZ Tissue Typing Laboratory; Nariman Aziz and David Wisternoff for collating patient replies and original request form information; the Molecular Genetics Department at Auckland Hospital for the use of their DNA sequencer; Jon Downing for assistance in calculating genotype and phenotype frequency; and Chris Darke for valuable assistance with statistical analyses.

Author contributions

This work was submitted as part of the requirement for Fellowship of the New Zealand Institute of Medical Laboratory Science. The author declares no conflicts of interest.

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Prevalence of macrocytosis in patients with chronic obstructive pulmonary disease

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Abstract

Background Macrocytosis is a common finding in the hospital and community setting, generally attributed to alcoholism, vitamin B-12 and folate deficiencies and certain medications. Only few studies have reported macrocytosis in chronic obstructive pulmonary disease (COPD).

Aim The aim of this study was to determine the prevalence of increased mean red cell volume (MCV) in COPD in an out-patient setting and to analyse its relationship to respiratory parameters.

Methods Ninety patients were retrospectively evaluated over a one year period. Patient demographics, smoking history, dyspnoea score, forced expiratory volume (FEV1) and haematological parameters were recorded from their medical charts. Macrocytosis was defined as a MCV $\geq$ 94 fl.

Results Twenty-one patients demonstrated macrocytosis giving a prevalence of 23.3%. Mean MCV (SD) of patients with and without macrocytosis was 97.5 fl (4.9) and 87.7 fl (7.5) respectively. FEV1 was significantly higher in patients with macrocytosis (p=0.03) There were no significant differences in body mass index (BMI), dyspnoea scores or current smoking status between patients with and without macrocytosis.

Conclusion Macrocytosis is a frequent finding in COPD patients attending an outpatient pulmonary clinic.

Key words: macrocytosis, chronic obstructive pulmonary disease, mean cell volume, prevalence

N Z J Med Lab Sci 2010; 64: 86-87

Introduction

Red cell macrocytosis has been frequently described in the hospital and community setting. Macrocytosis is commonly attributed to alcoholism, B12 and folate deficiencies, or certain medications (1,2). Less well known is that macrocytosis can be a finding in patients with COPD. One recent study has demonstrated that macrocytosis was present in almost half of hypoxic COPD patients (3), while another study demonstrated that macrocytosis was present in 29% of COPD patients without respiratory insufficiency (4).

Apart from those studies, there have only been a limited number of small studies describing macrocytosis in COPD patients. The primary aim of our study was to determine the prevalence of macrocytosis in stable COPD patients in an outpatient setting and to determine whether macrocytosis in that setting was associated with respiratory parameters.

Materials and methods

Study design and patients

We retrospectively reviewed the medical charts of all COPD patients (ICD-9 code: 496; chronic airway obstruction) attending the outpatient pulmonary clinic at Changhua Christian Hospital, Changhua City, Taiwan for a one year period (March 2008 to 2009). Excluded were patients with documented vitamin B-12 or folate deficiency and patients with a high alcohol intake.

From the medical charts we recorded the patients demographics (gender, age, weight, height, diagnosis), spirometry data (FEV1), smoking history (current smoker, ex-smoker for >12 months, never smoker), alcohol intake (non-drinker, occasional drinker, moderate drinker), modified Medical Research Council dyspnea score (5), and haematological parameters (red cell count, haemoglobin, haematocrit, mean cell volume and platelet count). Body mass index (BMI) was calculated by dividing weight (Kg) by height squared (M^2).

Spirometry and laboratory methods

FEV1 was determined on a MasterScreen CPX (VIASYS Health Care, Hoechberg, Germany) according to ATS spirometry standards (6).

Hematological parameters were determined on a Coulter LH 730 Haematology Analyzer (Beckman Coulter Inc., Fullerton, CA, USA). Macrocytosis was defined as a MCV of greater than 94 fl (4).

Statistical analysis

Statistical analyses were carried out using "R" version 2.9.1 (R Foundation for Statistical Computing, Vienna, Austria). Comparisons were made between COPD patients with macrocytosis (MCV $\geq$ 94fl) and those without macrocytosis (MCV < 94fl). Results are reported as the mean and standard deviation for both groups and p-values come from the Mantel-Haenszel non-parametric test. Odds ratios (OR) and 95% confidence intervals (95% CI) are from logistic regression. Statistical significance was set at p<0.05.

Results

A total of 90 COPD patients (71 male) charts were retrospectively reviewed in the 12 month period. Their demographics, lung function tests and haematological parameters are shown in Table 1. Six patients were current smokers, eight were never smokers and the remaining 76 were ex-smokers (> 12 months).

Twenty-one COPD patients (23.3%) had a MCV value of $\geq$94 fl of whom three had a MCV >100 fl. Results of those patients in comparison with patients whose MCV was < 94 fl are shown in Table 2. It shows that there were no significant differences in BMI or dyspnea scores while FEV1 was significantly lower in those without macrocytosis.

Table 1. Patients' demographics, respiratory function and haematological parameters

	Mean value	Range of values
BMI (kg/m^2)	22.9	15.8 – 32.8
Age (years)	73.3	49 – 94
FEV1 (% best predicted)	68.7	23.9 – 122.3
Red cell count (10^{12}/L	4.43	2.18 – 6.06
Haemoglobin (g/L)	134.8	86.0 – 167.0
Haematocrit (%)	39.5	25.0 – 47.7
MCV (fl)	90.0	53.6 – 115.0
Platelet count (10^9/L)	247	127 - 550

COPD patients with macrocytosis had significantly lower red cell counts, but no significantly differences in haemoglobin, haematocrit or platelet count levels. Although macrocytosis was more common in males than in females (OR: 1.23; 95% CI: 0.38-3.93) and current smokers compared to ex or non-smokers (OR: 2.33; 95% CI: 0.57-14.74), these differences were not statistically significant.

Table 2. Comparison of COPD patients with and without macrocytosis

	MCV > 94 fl	MCV < 94 fl	*p*
BMI (kg/m^2)	22.2 (3.2)	23.1 (3.9)	0.32
Age (years)	74.0 (8.4)	73.1 (10.7)	0.80
FEV1 (% best predicted)	77.6 (21.4)	66.0 (25.4)	0.03
Dyspnea score	1.14 (1.06)	1.57 (1.06)	0.11
Red cell count (10^{12}/L	4.10 (0.67)	4.54 (0.59)	0.01
Haemoglobin (g/L)	136 (20.0)	134 (15.8)	0.59
Haematocrit (%)	39.7 (5.9)	39.4 (4.6)	0.98
MCV (fl)	97.5 (4.9)	87.7 (7.5)	>0.0001
Platelet count (10^9/L)	238 (42.5)	250 (83.3)	0.58

Results are mean (1 SD).

Discussion

Our study has shown that 23.3% of stable COPD patients had macrocytosis, defined as a MCV of >94 fl. This compares to a prevalence of 29% in a previous study of stable COPD patients (3). In agreement with that study we also did not find differences in BMI, smoking status, or dypsnea score between COPD patients with and without macrocytosis.

In our study COPD patients without macrocytosis had significantly lower FEV1 levels and slightly, but not significantly, higher dyspnea scores. We found no significant differences in haemoglobin or haematorcrit between the two groups, unlike a previous study that found statistically significant lower haemoglobin and haematocrit levels in patients with macrocytosis (4). In that study COPD patients with hypoxemia had a much higher prevalence of macrocytosis (43.75%). However, the severity of hypoxia in that study did not correlate with the erythropoietic response (4). The authors of that study hypothesised that acute erythropoietic stress occurs repeatedly in COPD as a result of frequent exacerbations leading to release of relatively immature, large red blood cells from the bone marrow, as evidenced by a significant increased number of F-cells that were significantly correlated with MCV levels.

Apart from these two recent reports describing macrocytosis in COPD patients (3,4) there have been only a few earlier reports describing this association (7-10). The earlier viewpoint was that macrocytosis in COPD was due to acidosis or CO_2 retention. Current thinking is that it is likely due to enhanced release of large red blood cells from the bone marrow as a result frequent hypoxemic exacerbations (3,4). However, other unexplored factors may also be involved in the erythropoietic response to hypoxemia and need to be explored.

A limitation of our study is that we did not measure vitamin B-12 or folate levels in our patients, deficiencies of which are a common cause of macrocytosis. Some of our patients most likely did have anaemia, as evidenced by low haemoglobin and red cell counts. However, the prevalence of macrocytosis was similar to another study where patients with haemoglobin levels of > 130 g/L were excluded (4). Excluding the 32 patients with a haemoglobin level of > 130 g/L from our study only slightly reduced the prevalence of macrocytosis from 23.3% to 18.6%. We also did not record oxygen saturation and thus some patients may have had significant hypoxia. However, none of our patients were hospitalised, all attended an outpatient pulmonary clinic. If they were severely hypoxic they would most likely have been hospitalised.

In conclusion, macrocytosis is a frequent finding in COPD patients attending an outpatient pulmonary clinic. Many haematology text books and macrocytosis review articles fail to mention COPD as being associated with macrocytosis, instead focussing on vitamin B-12 and folate deficiencies, smoking, heavy alcohol intake and certain medications. Thus physicians and laboratory scientists have to be aware that a raised MCV in COPD may be due to factors other than the usual known factors associated with macrocytosis. Further studies are required to explore the likely complex factors involved in macrocytosis in COPD.

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Author contributions

Francis Wu and Mei-Wen Wu helped plan the study, collected the data and contributed to writing the article. Chin-Fu Chang helped plan the study and contributed to writing the article. Nevil Pierse conducted the statistical analysis and contributed to writing the article. Rob Siebers conceived and planned the study and was the primary author. The authors have no conflicts of interest to declare.

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Validation of the "OTHERS" parameter on the Sysmex XE-2100 as a predictor for the presence of activated B-lymphocytes in the peripheral blood

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The "OTHERS" parameter on the Sysmex XE-2100 is reported as both a percentage and an absolute value and numerically represents an abnormal leukocyte population with a high RNA content (1). Morphologically activated B-lymphocytes are lymphoplasmacytoid cells and plasma cells (2). The presence of activated B-lymphocytes in the peripheral blood occurs during an early response to infection (2). The aim of this project was to validate the "OTHERS" parameter as a measurement of activated B-lymphocytes in the peripheral blood, along with making recommendations to the departmental film review protocol.

Peripheral blood samples from 130 patients were analysed on the XE-2100. The "OTHERS" percentage values obtained for each sample were compared with a 100 cell differential performed using CellaVision DM96 and a manual 200 cell differential performed using light microscopy. It was ensured that all samples were from patients without haematological malignancies, because these patients may have leukocytes present in their peripheral blood which are not activated B-lymphocytes but are also present in the abnormal population that is reported as the "OTHERS" value (1). Samples from paediatric patients younger than two years of age were also not used in this project as activated B-lymphocytes, which appear in the peripheral blood, are activated in the marginal zone of the spleen and this region of the spleen is often underdeveloped until two year of age; as a result B-lymphocytes are not optimally activated (1,3).

Comparison studies with the "OTHERS" percentage value gave a poor correlation ($R^2 = 0.3173$) against the DM96, whilst a good correlation ($R^2 = 0.6673$) was found with light microscopy. Due to a gold standard such as flow cytometry being unavailable for use the XE-2100 was used as a reference method for sensitivity calculations. The sensitivities of the DM96 and light microscopy differentials for detecting activated B-lymphocytes were 32% and 69% respectively. However, when calculated using data from samples with an "OTHERS" percentage value of 1.0 or greater the sensitivities were 79% for the DM96 and 100% for light microscopy. Although activated B-lymphocytes were detected in the peripheral blood when the "OTHERS" percentage value was as low as 0.2%.

It was concluded that the "OTHERS" parameter can be used as an indicator for the presence of activated B-lymphocytes in the peripheral blood in individuals without haematological malignancies and in those greater than two years of age. Detection of activated B-lymphocytes in the peripheral blood is routinely done by examining blood films using a light microscope, however this method is time consuming and maybe inaccurate in part due to the presence of low numbers of activated B-lymphocytes in the peripheral blood (2). Therefore the acceptance of the "OTHERS" value as a reportable parameter would result in decreased turnaround time and increased accuracy for the quantification of activated B-lymphocytes. The presence of activated B-lymphocytes in the peripheral blood is a result of the first B-lymphocyte response to foreign antigen, therefore the "OTHERS" parameter could potentially become a useful diagnostic tool in differentiating between infection and inflammation (1). The "OTHERS" parameter may also aid in establishing the stage of infection as there is a greater elevation in activated B-lymphocytes during the healing phase compared with the acute phase, however, further studies are required to evaluate its usefulness (1).

Recommendations made to the film review protocol:

- When the "OTHERS" percentage value is 1.0 or greater there can be confidence that activated B-lymphocytes are present in the peripheral blood.
- The sensitivity of the "OTHERS" parameter in detecting activated B-lymphocytes in the peripheral blood may however be as low as 0.2%, but further studies are needed to confirm this. Therefore, the blood film should be scanned for activated B-lymphocytes when the "OTHERS" percentage value ranges from 0.2-0.9.
- When the "OTHERS" percentage value is 0.0 or 0.1 there can be confidence that either activated B-lymphocytes are absent from the peripheral blood or present in clinically insignificant numbers.

A 100 cell differential performed using the DM96 is routinely performed on blood films at Canterbury Health Laboratories, the results of this project show that this method of performing a differential has a low sensitivity for detecting activated B-lymphocytes. Therefore, it is recommended that peripheral blood films should be scanned for activated B-lymphocytes when the 100 cell differential performed using the DM96 is negative and the "OTHERS" percentage value is 0.2 or greater.

Acknowledgements

This project was conducted at Canterbury Health Laboratories as a requirement for the Bachelor of Medical Laboratory Science degree (University of Otago). We would like to thank the haematology staff at Canterbury Health Laboratories for their guidance of this project. A special thanks to Linda Henshaw for sharing her morphological expertise.

Author contributions

Emma Cochrane carried out the project and was the primary author. Kenneth Beechey conceived and supervised the project and contributed to writing of the article. The authors have no conflicts of interest to declare.

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Presidents report

Kevin Taylor

This year saw three changes in Council membership with Jackie Wypych from Hawkes Bay Hospital Laboratory and Anne Buchanan from SCL Kew, stepping down as the region three and five representatives respectively and Ken Beechey from Canterbury Health Laboratories being elected to the position of Vice President on council. The Institute greatly appreciates the valuable contributions that Jackie and Anne made during their time on Council, and would particularly like to highlight the work that Anne put into the QMLT examinations and the setting up of the Southern regional educational meetings. In light of these changes the NZIMLS council has been extremely lucky to be joined by three new representatives; Kim Allan from Aotea Pathology for region three, Tony Barnett from MedLab South, Nelson for region four and Terry Taylor from SCL Dunedin for region five.

After the deaths of two prominent members of the profession in 2008, both of whom had been previous Council members, the NZIMLS has established the Rod Kennedy and Barrie Edwards scholarships to honor the memory of these two medical laboratory scientists who contributed so much to the profession during their lifetime. These will be awarded twice yearly by the NZIMLS to a NZIMLS member to be able to attend and participate in an international conference.

During 2009 Council continued to promote the profession, with an emphasis on raising the profile of the profession amongst secondary school students. The members of Council believe strongly that the promotion of medical laboratory science, as an exciting career to students, will ultimately benefit the profession through improved recruitment. Enthusiastic and bright young medical laboratory science graduates are vital for the future of our profession. Efforts to promote the profession in this manner through the exhibition at careers expos has continued during the 2009 year and has also been picked up by numerous laboratories throughout New Zealand that are running a variety of "in house" professional promotional activities. Evidence to date suggests that there has been not only a marked awareness in the existence of medical laboratory science as a career option, but also a dramatic increase in secondary school students that display a passion and aptitude for science considering and choosing medical laboratory science as a valuable career option.

The NZIMLS has had a keen interest in the professional workforce and initiated a review of current professional numbers and likely scenarios of employable scientists within New Zealand in 2006/07. This showed that it was likely that total scientist numbers would decrease approximately 10 percent every five years unless proactive action was taken. It is with credit that through actions taken by the NZIMLS; through exhibiting at careers days and the segment for the Just the Job television series; NZIMLS members and medical diagnostic laboratories throughout New Zealand, that possible workforce crisis that may have presented in the future are now likely to be avoided.

The next challenge for medical laboratories and the medical laboratory science profession is the promotion of the value that medical laboratory scientists are able to provide to the patient care continuum. It is through increasing awareness of the value added contributions made by medical laboratory scientists to the health care system that will see an improved recognition of medical laboratory scientists. This was perhaps highlighted through the H1N1 pandemic, where the integral role played by medical laboratory scientists was often overlooked, not only by the general public, but even within the healthcare profession. To assist in increasing awareness and to continue the work that the NZIMLS has already initiated in professional promotion to secondary school students, the NZIMLS has employed a Communications Officer. Keeley Eastwood, the NZIMLS communications officer, joins the NZIMLS from Trade Aid Importers, New Zealand's not for profit fair trade organization, but has also had two years experience as the sole Press Officer for the British Epilepsy Association.

The NZIMLS has continued to provide QMLT examinations for the trainee medical laboratory technicians. Reviews of the QMLT examination processes has seen the council initiate a number of changes which are to be introduced into all QMLT disciplines. These include the provision compulsory logbooks for all disciplines and a restructuring of the examination format. The introduction of logbooks has been initiated as the QMLT examinations are a form of competency assessment, and it is important that successful candidates have their technical skills at an appropriate standard in addition to being able to display theoretical competency that has been previously assessed, and will continue to be, through examination. The NZIMLS has continued its efforts to enhance the standard of examinations through the provision of workshops for QMLT examiners and moderators. It has been highlighted through post examination reports from examiners and moderators that there are areas for improvement in key aspects of knowledge of candidates that are sitting exams and the need for consistency across disciplines. To improve in this area the QMLT exams will have a set format, with minimum percentages of the examination paper consisting of key aspects of the profession (e.g. the ability of the candidate to perform calculations).

Despite global financial constraints the NZIMLS has managed to maintain its financial position without having to increase either membership fees or CPD enrollment costs. This has largely been through improved financial management of educational events run under the auspices of the NZIMLS. Although the attendance of the annual scientific meeting held in Blenheim had lower attendance than in recent years, regional meetings and special interest group meetings have continued to have strong attendance and reflects well on both the organization and willingness of NZIMLS members and the profession to aid and contribute to these seminars.

At the annual scientific meeting in Blenheim, the NZIMLS council recognized the fantastic contribution made to the NZIMLS by its Executive Officer, Fran van Til, and recognized this by awarded her life membership of the NZIMLS.

Finally, I would like thank my fellow Council members for the effort that they have all put in on your behalf during the year. Also, it is appropriate to acknowledge the many Institute members who have unstintingly given of their own time to the development of the profession in numerous ways.

New Zealand Institute of
Medical
Laboratory
Science

Minutes of the 66th Annual General Meeting held at the Copthorne Hotel & Resort, Paihia on Thursday 26th August 2010 at 4.40pm

PRESENT:
The Secretary / Treasurer presided over approximately 40 members.

APOLOGIES:
Motion:
Moved K Allan, seconded F Taylor
That apologies be accepted from K Taylor, K Beechey, S Woods, M McCarthy, B Kendrick, P Wakem, M Hammond.
Carried

PROXIES
Motion:
Moved K Allan, seconded M Legge
That the list of four proxies be received.
Carried

MINUTES OF THE PREVIOUS ANNUAL GENERAL MEETING
Motion:
Moved K Allan, second J Broadbent
That the minutes of the 65th Annual General Meeting held on 20 August 2009 be taken as read.
Carried

Motion:
Moved R Siebers, seconded C Kendrick
That the minutes of the 65th Annual General Meeting be accepted as a true and correct record
Carried

BUSINESS ARISING FROM THE MINUTES
Nil

REMITS AS CIRCULATED

Motion:
Moved R Hewett, seconded K Allan
That the following Remits be accepted.
Carried

1. "THAT Policy Decision Number 1 be reaffirmed"

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

2. "THAT Policy Decision Number 2 be reaffirmed"

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

3. THAT an amendment be made to the NZIMLS Rule 13(h) that a by-election or secondment can occur when there is a vacant position on Council.

   The Rule 13(h) to read:
   "If any vacancy shall occur in the Council of the Institute, such vacancy may be filled by Council either by secondment or through a by-election and any person so appointed shall hold office until the next Annual General Meeting of the members of the Institute."

4. THAT the subscription rates for membership be adjusted from 1st October 2010 to be:

Members	$114.50
Associates	$57.25

   Explanation
   The increase in subscriptions is necessary to meet the increased cost of GST and this will be reflected in the 2011 membership invoices. Those applying for membership or for those who have not paid their membership as at 1st October, this new rate will apply.

PRESIDENT'S REPORT
Motion:
Moved R Hewett, seconded C Kendrick
That the President's Report be received.
Carried

ANNUAL REPORT
Motion:
Moved R Hewett, seconded F Taylor
That the Annual Report be received.
Carried

FINANCIAL REPORT
Motion:
Moved R Hewett, seconded R Siebers
That the Financial Report be received.
Carried

ELECTION OF OFFICERS
The following members of Council were elected unopposed:

President:	K Beechey
Vice President:	C Pickett
Secretary/Treasurer:	R Hewett
Region 3 Representative:	K Allan

Region 4 Representative: T Barnett
Region 5 Representative: T Taylor

The results of the elections for:

Region 1 Representative:	L MacDonald	60
	M Matson	111

Therefore, the Region 1 Representative will be M Matson.

Region 2 Representative:	J Bird	51
	MA Janssen	41

Therefore, the Region 2 Representative will be J Bird.

Motion:
Moved R Hewett, seconded H Perry
That the election of officers be approved.
Carried

PRESENTATION OF AWARDS

The award winners were announced and the following awards were presented by the Chairman:

Qualified Medical Laboratory Technician Awards
Clinical Biochemistry – Elizabeth Jones, Canterbury Health Laboratories
Haematology – Marjorie Martinez, Diagnostic Medlab
Histology – Shalini Gajadhar, Capital & Coast DHB
Immunology – Olivia Logan, Aotea Pathology
Microbiology – Korreen Hay, Southern Community Laboratories, Hawkes Bay
Transfusion Science – Tanya Arthur, Labcare Pathology
Transfusion Science Blood Products – Sarah Walsh, New Zealand Blood Service, Wellington
Mortuary Hygiene & Technique – William Little, Southern Community Laboratories, Invercargill

Qualified Phlebotomy Technician
QPT – Christina Stanbridge, Diagnostic Medlab

Qualified Specimen Services Technician
QSST – Daniel Millar, Medlab Central

HONORARIA
Motion:
Moved R Siebers, seconded J Broadbent
That no honoraria be paid.
Carried

AUDITOR
Motion:
Moved R Hewett, seconded C Pickett
That Hilson, Fagerlund Keyse be appointed as the NZIMLS auditors for the 2010/2011 financial year.
Carried

GENERAL BUSINESS
John Elliot, on behalf of PPTC, thanked the NIZMLS for their donation of $6,000 this year to the PPTC.

VENUE FOR THE 2011 CONFERENCE
This is a combined Australian Institute of Medical Scientists and NZ Institute of Medical Laboratory Scientist, South Pacific Congress to be held on the Gold Coast, 8-12 August 2011.

2012 CONFERENCE
This potentially will be a combined conference with the Australian Association of Clinical Biochemists at the Christchurch Convention Centre, 27-31 August 2012.

Meeting closed 5.10pm

Fellowship of the New Zealand Institute of Medical Laboratory Science

The NZIMLS encourages members to consider Fellowship as an option for advancing their knowledge and career prospects. Fellowship provides an attractive option to academic postgraduate degrees at a fraction of the cost.

Fellowship of the NZIMLS may be gained by examination, by thesis or by peer-reviewed publications.

Examination
Consists of two parts:

a) Part 1: Two written papers each of three hours duration
b) Part 2: Upon successful completion of Part 1 a dissertation of 3000 - 5000 words

The dissertation may take the form of a review, development of a hypothesis or any other presentation that meets with the approval of the Fellowship Committee.

Thesis
The thesis must be based on the style of Master of Science by Thesis requirements of New Zealand Universities and not exceed 20,000 words.

Publications
A minimum of seven peer-reviewed publications, of which the candidate must be first author of at least four, may be submitted for consideration. These need to have been published in international or discipline acknowledged scientific journals. A review of the submitted articles of 3000 – 5000 words must also be submitted. The candidate must state the contributions he or she made to the publications.

Exemption
Candidates who are holders of postgraduate qualifications in Medical Laboratory Science (academic or professional) may be exempt from the Part 1examinations but are still required to submit a dissertation for Fellowship.

Qualifications recognised by the NZIMLS for the purpose of exemption to sit the Part 1 examinations are:

- Fellowship of the Australian Institute of Medical Scientists (FAIMS), the Institute of Biomedical Science (FIBMS) and the Australasian Association of Clinical Biochemists (FAACB)
- An academic postgraduate qualification in medical laboratory science. The course of study must meet the minimum requirement of one year's full-time study

For full Fellowship regulations and application process visit the NZIMLS web site: www.nzimls.org.nz

GOLDCOAST 2011

AIMS NZIMLS SOUTH PACIFIC CONGRESS

LIGHTS
CAMERA
ACTION

AIMS NZIMLS
South Pacific Congress
5-8 August 2011
Gold Coast Convention Centre
Queensland Australia

The Australian Institute of Medical Scientists and the New Zealand Institute of Medical Laboratory Science is proud to host and invite you to the South Pacific Congress, 5-8 August 2011. The Congress will bring to the Gold Coast Convention Centre a top level forum of leading national and international speakers to address topical issues in the medical science industry.

Keynote speakers include:

- **Dr Barbara Bain** (UK)
  Haematology
- **Associate Professor Mark Sheppard**
  POC
- **Dr Robert Webb**
  Director, Hyperbaric Medicine Service
- **Carol Turnbull** (UK)
  Anatomical pathology
- **Professor Peter Rathjen**
  Stem cell research

The Congress theme **'Lights! Camera! Action!'** has been chosen as a call to action for delegates to spend a focussed 4½ days in the vibrant Gold Coast at a Congress filled with topical and relevant presentations. Daily sub-themes will logically group presentations and have a little fun based on the Congress theme!

- 'Waterworld' – Water trauma/diseases
- 'Basic Instinct' – Back to basics
- 'Aliens' – The immune system
- 'Back to the future' – New technology
- 'That's all folks!' – Closing day

In the spirit of the joint AIMS and NZIMLS South Pacific Congress, the Inaugural Bloodisloe Cup held on the final day of the Congress, will be an event not to be missed!

Be Noticed – Become a Sponsor!
Sponsorship opportunities are available for organisations interested in this important Congress. Please contact All Occasions Management for more information.

FOR FURTHER INFORMATION ABOUT THE CONGRESS PLEASE CONTACT:
ALL OCCASIONS MANAGEMENT 41 ANDERSON ST THEBARTON 5031 SOUTH AUSTRALIA
T. +61 8 8125 2200 F. +61 8 8125 2233 E. CONFERENCE@AOMEVENTS.COM
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Abstracts of oral and poster presentations at the Annual Scientific Meeting of the NZIMLS, Bay of Islands, Northland 2010

Editor's note: Only abstracts that contain data or facts are shown. Any abstract containing the phrase "*results will be presented*" or "*results will be discussed*" are not listed or have had those phrases removed.

Rawene health initiative. Diagnostics in Hokianga: avoiding insomnia in a laboratory free zone

Dr Clare Ward, Kati Blattner, Hokianga Health Enterprise Trust

Hauora Hokianga is a community owned and governed organization providing integrated health care to a rural/remote high needs population. This includes Rawene Hospital which is two hours from the closest Base Hospital at Whangarei. Hokianga Health is the sole provider of health services for the Hokianga community and in addition to GP/community care also provides 24/7 acute services from Rawene Hospital which is the local A&E and also has ten in-patient acute beds as well as three maternity beds.

The local clinicians manage patients with the same problems as those presenting to any city hospital. However, in contrast to city hospitals, we do this with very limited lab diagnostic services. A POCT analyser was introduced into Rawene Hospital in 2008. We have carried out a research project looking at the impact that this has had on the community and on the clinicians. Having basic lab tests available, especially for acutely unwell patients has led to improved decision making and hence improved the confidence of local clinicians in managing these patients.

Role of the reference laboratory

Ross Hewett, LabPlus, Auckland

The reference laboratory is a multi-facet organisation with different service provisions for a variety of users. From research to routine, clinical trials to point of care, the complexity is often confusing and finding the right staff is difficult. This is because the lab has to find both a high number of specialist scientists and those able to be multi-skilled who rotate through the sections and perform routine work, 24 /7. The blending of these two distinct groups can often lead to difficulties in career progression, fixed or silo attitudes, elitism and managing change.

However such diversity also creates opportunities for those who have the desire to progress and create a career in Medical Laboratory Science. The complexity offered in a reference laboratory can also lead to further studies at post-graduate level in science, research, IT and management. The ever changing world of technology creates new opportunities for development as the unusual or obscure tests become routine and no longer stay in the exclusive domain of the reference laboratory.

Remisol middleware

John Woodford, Pathlab Waikato

Remisol is a middleware program developed in France and supplied by Beckman Coulter. It allows up to three analysers to use a single bi-directional interface to the Laboratory Information System (LIS). Multiple Remisol clients can be linked to a Remisol server to accommodate larger numbers of analysers. Sample requests and results are processed using 'rules based programming' and the system is fully auditable.The user programmable software enables laboratory staff to manage and control laboratory processes and bring about standardisation of practice. A comprehensive Quality Control package is also available.

In Pathlab Waikato Biochemistry department, we process over 3000 samples per day generating over 30,000 results. The rules within Remisol automatically validate 97.5% of results, perform complex delta checks and generate commands to the LIS to 'auto-fax' results outside of defined limits. Rule based generation of comments and messages give direction to staff and facilitate standardisation of practice. Results processing by the LIS is now much less complex, as only reportable results are received from Remisol. Change to the Remisol configuration can be made by laboratory staff immediately, without the involvement of IT staff, allowing us to adapt to changes in demand.

The challenges, surprises & experiences involved in becoming a multiskilled MLS in a Northland DHB from an African migrant's perspective

Noah Mhlanga, Northland DHB

Migrating to work in a country with a different cultural as well as technological differences comes with a fair amount of challenges but great opportunities for professional development. My name is Noah Mhlanga and I am 36years of age, married with two children. Before I came to New Zealand I was working at a referral Hospital in Gaborone Botswana a neighbouring country to where I come from. I am Zimbabwean and I did in my education at the University of Zimbabwe Medical School. I qualified with a General Diploma in Medical Laboratory Sciences and went on to do a Specialist Diploma in Immunology. I have worked mostly in referral Hospitals where one gets to work in one area of specialty. Coming to New Zealand at NDHB I am employed as a Scientist at Kaitaia Hospital Laboratory where all of us are expected to multiskill and work in all the departments in the Laboratory. The main challenge from a technical point of view has been to upskill in all the departments to a level where I can comfortably work without supervison it's been a year since I worked in Kaitaia I must say its been a great journey. The academic and practical experience I have acquired over the years has made it a lot easier fro me to adapt to my work situation, because after the general diploma qualification in my country one is acquinted with working in most of the departments the only challenge I have faced is upskilling in areas I have not really worked in for a long time. From a social point of view living in Northland has been great for both me and the family, we have met great people with big hearts who have helped welcome us into our new home in New Zealand. I confidently say the NDHB can continue to hire from Africa without fear because of excellent grooming and grounding that we get during our training. These are all part of the challenge which makes the work real and relevant.

The multi-functional role of the community based hospital scientist

Jackie Wright, Ashburton Hospital

Purpose: To determine which laboratory role is the most fulfilling and challenging.

Method: 28 years of laboratory work was undertaken in a variety of laboratory settings.

Results: 1 x tertiary laboratory: five years; 1 x reference laboratory: three years, 1 x food laboratory: two years; 3 x community-based hospital laboratory: total 18 years.

Conclusion: The work in a community-based hospital laboratory is fast paced, varied, and relevant. In the course of one day your role ranges from phlebotomist to clinical educator; from patient registration to result evaluation and interpretation; from telephonist to analyser engineer; from manual review to blood film review; from caterer to quality officer. Each day you are reminded how much more you have to learn; and each day you remember why you are here as you go face-to-face with both patients and clinicians. There is no "them and us": you are part of a laboratory team which is part of a clinical team and you know you are appreciated by both. Sure, there are bad days - the days where you just can't seem to find the veins "that no-one else has ever had trouble with"; the days when a change is foisted upon you which you are initially unwilling to accept; the days when a patient who you have come to know passes away. These are all part of the challenge which makes the work real and relevant.

The problems of atrophy

Nick Dudding, Sheffield Teaching Hospitals, East Pennine Cytology Training Centre, UK

Atrophy is defined in my dictionary as a decrease in size of a body organ, tissue, or part owing to disease, injury, or lack of use. To the cytologist it relates to the decrease in size of the uterus and of the thickness of the squamous epithelium that results from the reduction in oestrogen and progesterone production when a woman enters the menopause. Another definition would be "a difficult sample"!

Whilst the majority of atrophic samples we encounter are related to the menopause, we can see similar patterns in younger women, during the post natal period and, heaven forbid they ever be taken, prior to menarche. It is also possible to observe atrophic patterns in pre-menopausal women following the use of progesterone dominant contraceptives such as depoprovera, following prophylactic use of tamoxifen and, without the use of hormone replacement therapy, in women who have had irradiation of the ovaries. Perhaps less well known is that stress, depression, or even anorexia nervosa can also result in the presentation of an atrophic pattern.

Atrophic patterns are, of course, highly variable and the onset of the menopause rarely sudden and whilst it might be expected that women of 64 years might present with a more atrophic pattern than one of 51, this is not always the case. Why is atrophy problematic? The two basic problems with the atrophic sample are screeners either missing abnormalities or overcalling negative samples as abnormal. The major reason for this lies with the fact that in atrophy abnormal and normal cells can appear frighteningly similar.

Missing abnormalities: In a mature sample from pre-menopausal women those screening the sample are minded to inspect any group of cells or single cells with disproportionate nuclear enlargement. i.e. a high n/c ratio. In fact, over time, this is exactly what we have trained our eyes to do. Of course all parabasal cells fulfil this description and so the contrast between normal and abnormal is minimal. This severely challenges the visual systems of the human eye. Such challenges are exaggerated by the fact that whilst atrophy is not especially rare, dyskaryosis is this age group is not especially common. English statistics show that only 8.6% of our dyskaryotic samples present in women over 50 years of age (1). Thus the old adage; what is often not sees, is often not seen comes into play with the screener expecting the slide to be normal fulfilling their own expectations. To add to the problem there are cases where it practically impossible for the human eye to identify abnormal cells. Where the background is severely atrophic or when we see atrophic cervicitis this is particularly true. In addition, to these problems of visual search screening is made more complicated by the high no of degenerate cells and tightly packed groups. If you are using LBC, particularly Surepath™, the problems will be emphasised by the superb fixation which highlights every degenerative notch and fold. If you are using conventional smears you will have the problems of are drying and trauma. After five years of experience with LBC those of you still using conventional samples have my sympathies!

Overcalling: The flipside of missing abnormalities is the problem of overcalling and a disproportionate number of samples passed to me by screeners come from menopausal women. The problems arise because of some of the issues noted above, but are inextricably linked to the confidence of those doing the screening and those lacking confidence / experience will always find atrophic samples more difficult. Interestingly, just as stated with missing abnormalities, the fact that we don't see dyskaryosis in this age group very often adds to the problem. If screeners saw dyskaryosis in atrophy every day they would soon become more confident. It is of note that individuals attending the atrophic workshops at the East Pennine Cytology Training Centre often arrive announcing that they find atrophy difficult only to find that after a day of lectures, a multiheaded microscope session and a slide workshop they do absolutely fine in the final self assessment section! As well as problems of confidence overcalling can also occur with specific entities such as "red atrophy" "blue blobs" transitional cell metaplasia and with SurePath™ the presence of "spikey cells".

Diagnostic pointers: Because all CIN represents a prevention of normal maturation dyskaryosis in atrophic samples is generally high grade. When encountered low grade changes, usually from condylomatous lesions, easily stand out and prevent little challenge. High grade changes are different. With both LBC and conventional smears many cases present as groups. The secret is to investigate any over-crowded groups on high power, particularly when the sample presents with a distinct two cell population. Be aware of relatively geometric shaped sheets with steep sides and sharp, straight edges. Once sheets are scrutinised on high power it is usually possible to get the right answer. If you see nuclei with clearly abnormal chromatin it is straightforward. If this is absent do not assume the cells are benign, particularly with conventional samples when air drying can obliterate any chromatin abnormality. Learn to rely on other features. In particular be very wary of disorganisation and a loss of polarity within the sheet. Are nuclei running in different directions? Are they overlapping and disrespectful of each others space? Normal sheets usually have nuclei that sit in their own space and run largely in the same direction. Look for pleomorphism; if the nuclei start to vary in shape and especially size, then think dyskaryosis. If one or two nuclei in the group are two / three times the size of their neighbours this is of great concern. Do look at nuclear margins, but beware. As stated above, degeneration is a big problem with atrophic samples. My favourite pointer is mitosis. Whilst inconclusive in younger women, I never accept their presence in atrophic samples. This epithelium is in retirement and so any mitotic activity needs an explanation. With LBC I have become obsessive about sheets, but single cells are just as important and again irregularities of outline, chromatin etc is extremely useful. One nuclear feature of benign cells that is often overlooked is that

benign nuclei generally have small chromocentres. The problems of the post natal sample are really the same as those listed above, but in my experience dyskaryosis (for some reason) is even rarer in these samples.

Reporting samples: Despite the difficulties of reporting these samples one must take care not to over use the borderline / borderline- inconclusive category. If you are genuinely uncertain a repeat after local oestrogen can be useful. In my practice we have the luxury of HPV testing (HCII) which is particularly useful in this age group because of the low background HPV rates and therefore higher specificity. In the UK the future the combination of LBC, HPV triage and even the introduction of molecular markers such as p16 (MTM Laboratories http://www.mtmlabs.com/cda/home.html) and ProExC (Becton Dickinson http://www.bd.com/anz/) should mean that our diagnostic accuracy can approach very high levels.

Reference

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Biochemistry and clinical relevance of new folate and vitamin B12 biomarkers

Dr Markus Herrmann, Mortlake, NSW, Australia

Folate and vitamin B12 deficiencies are common risk factors for various medical conditions including neural tube defects and neurodegenerative disease. B-vitamin deficiencies are also the leading cause of hyperhomocysteinemia in adults. Hyperhomocysteinemia is an independent risk factor for diseases such as stroke, cardiovascular disease, thrombosis and osteoporosis. The measurement of folate and total vitamin B12 levels however is unreliable, as the sensitivity and specificity of these assays are limited. The introduction of routine assays for the measurement of holo-transcobalamin II, methylmalonic acid and homocysteine has greatly improved our understanding evaluatioin of folate and vitamin B12 deficiencies. Traditional folate and total B12 assays reflect circulating concentrations of these vitamins. Only 20-30% of circulating vitamin B12 however is biological active. Furthermore, these assays do not provide information about the intracellular availability of folate and vitamin B12, which in patients with renal impairment or vitamin B12 receptor defects is often reduced. Holo-transcobalamin II represents the biologically active fraction of circulating vitamin B12 and has been found to be a better marker of vitamin B12 status than serum total vitamin B12 concentration. Methylmalonic acid is a metabolite in the transsulfuration pathway, which accumulates when intracellular B12 is low. It is therefore considered a functional marker of vitamin B12 status. The metabolization of homocysteine is mainly controlled by two enzymes requiring folate, vitamin B12 and B6 as co-enzymes. Homocysteine appears to be a reliable functional marker of folate, vitamin B12 and B6 status. Using these new parameters in clinical practice can improve diagnosis, treatment and management of patients.

Ferroportin mutations: ironing out the phenotype

Dr Neil van de Water, LabPlus, Auckland

Iron is essential for most forms of life including human beings. It plays a critical role in the transport of oxygen by haemoglobin and the average male adult has about 4g of iron in their body, predominantly within the red blood cells. Iron levels must be strictly regulated because too much iron will result in tissue and organ damage and too little iron will result in cellular dysfunction and anaemia. There is no effective mechanism for the excretion of excess iron and therefore regulation of absorption via the intestine must match systemic iron requirements. Many genes/proteins play an important role in the regulation of iron but when these genes become defective this can lead to iron overload disorders such as haemochromatosis. The majority of haemochromatosis patients (>85%) have a defect in their HFE gene. Mutations within the ferroportin gene are the second most common cause of haemochromatosis but can present with two different phenotypes. A "loss of function" mutation results in high ferritin but low transferrin saturation and iron accumulation in macrophage like cells. Other mutations result in a "gain of function" with high ferritin and transferrin saturation and accumulation of iron in hepatocytes. Insights into the structure and function of ferroportin can help to iron out which mutations will produce which phenotype.

FVIII mutation – a twist of a tail

Dr Neil van de Water, LabPlus, Auckland

Factor VIII (FVIII) is a cofactor for Factor IXa in the activation of factor X, and a deficiency of FVIII results in haemophilia A. The active FVIII protein is composed of three A domains and two C domains. Recent 3-dimensional structures of the FVIII molecule indicate that the C1-C2 tail of FVIII is twisted around and in a different orientation to previous 3D models. The new C1-A3 interface contains a number of hydrophobic residues which have been suggested to be important for holding this structure together. But just how important are these residues? Naturally occurring mutations in the FVIII gene provide an experiment of nature which may give insight into structure and function of the molecule. We have identified four mutations; I2080N, P2142S, P2142L and I2145M within the C1 domain and all lie along the C1-A3 interface.

GEAN workflow in the molecular genetics laboratory

Heather Sharman, South Eastern Area Laboratory Services, Sydney, Australia

Molecular genetics is moving away from being a 'boutique' laboratory service to a high throughput service, much the same as in a diagnostic clinical chemistry laboratory. This is a completely different way of working - for both the scientist at the bench and those in management roles. Genetics has started to move from "one scientist - one test" using manual protocols to "one scientist - many tests" using automation with First In First Out (FIFO) procedures.

Following the success of the LEAN pilot project in South Eastern Laboratory Services (SEALS) Central Specimen Reception in Randwick, the Genetics Department volunteered to be the next involved in LEAN. This Department wished to reduce Turn-Around-Times (TAT) for processing of molecular and cytogenetic samples, reduce the number of processing steps for front-end processing of genetic specimens and improve the laboratory layout whilst maintaining a high level of staff engagement. This journey is still a long way from complete but there have been encouraging results along the way.

Role of innovation in driving quality improvement in the pre-analytical phase of clinical laboratory practice

Brian Smith, BD Diagnostics Preanalytical Systems, Asia Pacifc, Japan and Eastern Europe, Middle East and Africa

Advances over the past two decades in analytical technology in the clinical laboratory have delivered significant improvements in terms of the sensitivity, specificity, accuracy and precision performance of a large array of diagnostic tests. Concurrent with these advances has been steady progress with analyser throughput, cost per test, quality assurance and quality control. Whilst advances in analytical methods and instrument platforms have placed greater demands on specimen quality, advances in the pre-analytical phase have been less evident over the same time period. Implementation

of effective quality improvement systems to assist in the reliable delivery of specimens free of pre-analytical artefact remains as a key challenge in many laboratories. With the pre-analytical phase accounting for more than half the labour costs and processing time in most laboratories, there is increasing awareness that future significant gains in overall quality of laboratory services are likely to come from improvements in the pre-analytical phase.

Glandular neoplasia and borderline endocervical reporting rates before and after conversion to a liquid based cytology system

Nick Dudding, Sheffield Teaching Hospitals, East Pennine Cytology Training Centre, UK

Background: Anecdotal evidence from two laboratories in the North East and Yorkshire Humber area in the UK suggested that reporting rates for glandular lesions, both endocervical and endometrial, had increased following conversion from conventional cytology to the Surepath™ liquid based cytology (LBC) system.

Methods: To assess whether this was true, a large scale audit across the North East, Yorkshire and The Humber Region was undertaken and all laboratories in the region that were able to submit 12 months' data, pre- and post-conversion to LBC were invited to participate. Workload data and reporting rates for glandular neoplasia during the last 12 months of conventional sampling and screening were compared with those achieved over a 12 month period of LBC processing starting six months after conversion had been completed.

Results: On conversion to SurePath LBC, the overall number of glandular abnormalities recorded across six UK laboratories increased from 83 to 117, with the rate per 10,000 samples increasing by over 65% from 3.4 to 5.6. Within this total increase, the number of endocervical lesions reported rose from 54 to 86, corresponding to an 80% increase in rate per 10,000 samples from 2.2 to 3.9. These statistically significant increases in the reporting rates were accompanied by Positive Predictive Values (PPV) which remained largely undiminished. Those reported as suspected endometrial or other origin showed a more modest increase from 29 to 31 cases: an increase of 20% in rate per 10,000 samples from 1.2 to 1.4. There was also an unexpected reduction in the number of reported cases of borderline changes in glandular cells which only just fell short of statistical significance.

Conclusions: The implementation of Surepath™ LBC into six laboratories in the North of England has resulted in significantly increased detection of endocervical glandular neoplasia and appears to have improved specificity of reporting by causing a small, but non-significant, decrease in the number of equivocal or borderline glandular reports.

National screening programmes

Jane McEntee, Dianne Webster, National Screening Unit, NTC, LabPlus, Auckland

Purpose: The aim of health screening is to reduce the morbidity and mortality of certain conditions as economically (in human and financial terms) as possible.

Methods: An overview of the theoretical and practical aspects of screening in New Zealand will be presented, with focus on newborn baby metabolic screening and antenatal screening for Down syndrome and other conditions and on the public health implications of screening programmes.

Results: All of us participate in screening at one time or another. Many of us are involved in providing screening services. Screening demands more stringent quality requirements than a lot of other health interventions. Successful screening, i.e. achieving health gains for a population, involves complex interactions between healthcare providers working in the different steps of the screening pathway i.e. offering screening, obtaining consent, ensuring testing occurs appropriately, giving results to the screened individuals, referring for diagnosis and treatment in a timely way as required. High quality testing is essential to ensure maximum benefits and minimum harms from screening. Laboratories and laboratory information systems have key roles in sample collection, testing, providing results and their interpretation, and increasingly in providing patient based information to enable programme evaluation and audit.

Conclusions: It is likely that more screening will be introduced over time and that the role of laboratories in screening will expand to include more holistic interactions with funders and the community. Laboratories must be prepared to meet this challenge.

Introduction to inborn errors of metabolism

Dr Callum Wilson, Starship Hospital, Auckland

Inborn errors of metabolism refer to genetic disorders of metabolic pathways that cause disease. The conditions are frequently due to a deficiency of an enzyme which then results in an accumulation of a toxic substrate or a deficiency of an energy related product. While the conditions can present at any age with any symptom they tend to present in early childhood and symptoms of liver, muscle and especially brain dysfunction are most common. The symptoms of metabolic disease can be general, such as developmental delay or jaundice although there are some symptoms such as neonatal encephalopathy, developmental regression or severe hepatomegaly that are very suggestive. Biochemical investigations including hypoglycaemia, acidosis, and transaminasemia may suggest metabolic disease but it usually requires specific laboratory biochemical tests such as amino acids, organic acids or an acylcarnitine profile to make the correct diagnosis. The diagnosis is then confirmed with a specific enzyme or molecular investigation. The conditions while all individually very rare are collectively not uncommon.

Tools and techniques for diagnosis of inborn errors of metabolism

Dr Kevin Carpenter, NSW Genetic Service, Australia

Inborn Errors of Metabolism (IEM) are a large group of genetic disorders involving a defect in the processing of intermediary metabolism. Often they are inherited as autosomal recessive conditions with carriers having no clinical or biochemical features. Where the defect involves an enzyme the result is usually an accumulation of the substrate and deficiency of the product, either or both of which may have toxic effects.

Traditionally the IEM have been grouped according to the area of metabolism affected; amino acid disorders, organic acid disorders, carbohydrate metabolism disorders etc. In terms of their laboratory diagnosis the techniques used have broadly followed these groupings. For example, amino acid chromatography or electrophoresis in plasma or urine is used to detect a number of different defects in amino acid metabolism.

The discipline of Biochemical Genetics is the area of laboratory medicine which deals with these conditions and the growth in the field has mirrored the improvements in separation techniques available (thin layer, paper, liquid and gas chromatography, cellulose acetate and capillary electrophoresis, mass spectrometry and hybrid techniques).

Global and local epidemiology of ESBL-producing organisms: the role of NZ laboratories in monitoring the ESBL epidemic

Dr Joshua Freeman, LabPlus, Auckland

Extended spectrum beta lactamase-producing Enterobacteriaceae (ESBL-E) are resistant to all beta lactam antibiotics apart from the carbapenems. Frequently, ESBL-E also exhibit resistance to the aminoglycosides, fluoroquinolones and the sulphonamides, leaving few treatment options. It is of concern therefore, that the global incidence of infection due to ESBL-E has increased dramatically over the last decade. ESBL genes of the CTX-M class in particular, have disseminated widely, not only within the ICU setting, which has been traditionally associated with ESBL, but also within the community, among patients who have had minimal prior exposure to healthcare services. Thus the "CTX-M beta-lactamase pandemic" poses a unique set of challenges for general practitioners and hospital physicians as well as professionals in infection control and public health.

In New Zealand and worldwide, CTX-M-15 and CTX-M-14 are the most prevalent ESBL gene types. Organisms carrying these genes have been associated with community-onset infections and recent travel to areas endemic with ESBL-E. Also among patients admitted to Auckland City Hospital (ACH), patients with CTX-M-15 producing ESBL-E have been found to have very different demographics from patients with CTX-M-14, suggesting that these two ESBL genes disseminate independently among different communities within the ACH catchment area.

ESBL-E should be considered a major public health issue in New Zealand. Diagnostic microbiology laboratories and national antibiotic reference laboratories such as ESR play an essential role in surveillance for ESBL infections and in the provision of data on which rational strategies can be based to prevent infections with ESBL-E.

Carbapenemases - are we doing enough?

Julie Creighton, Canterbury Health Laboratories, Christchurch

The last decade in New Zealand has seen a steady increase in both the frequency of multi-drug-resistant-organisms and in the number of different types of resistance mechanisms. Due to global spread and wide interspecies dissemination, Carbapenemases are the hot new topic. Carbapenemase resistance may be associated with resistance to many ß-lactamase and non- ß-lactamase antibiotics and leave very few treatment options. They often affect the most vulnerable patients and have higher associated mortality rates. Very few have been reported in New Zealand to date. But are we looking hard enough?

Many of the carbapenemases only weakly hydrolyse carbapenems, making them difficult to detect phenotypically. Carbapenemases are an efficient resistance mechanism, conferring multiple resistances which are a challenge for laboratories, clinicians and infection control.Early detection and prevention of spread are critical.

Antibiotic resistance detection - a technical update

Adrienne Coates, LabPlus, Auckland

The emergence of antibiotic resistance in previously sensitive organisms necessitates correct isolation and detection procedures for successful treatment outcomes and infection control. Vancomycin resistance in Enterococci was first seen in NZ in August 1994. Numbers remained low until an outbreak at Auckland City Hospital in 2007-2008. This was brought under control through carefully managed screening and infection control measures. However, correct identification and differentiation of the various Vancomycin resistance genes among the Enterococci pose some problems.

Plasmid-mediated AmpC beta-lactamase producing Enterobacteriaceae are being increasingly reported. They also have important implications for patient treatment and infection control as this resistance mechanism can be readily transferred on plasmids between bacteria of the same and different genera. There are currently no CLSI approved methods to detect plasmid-mediated AmpC beta-lactamase producing organisms. Currently we test *E.coli*, *Klebsiella* sp, *Proteus mirabilis* and *Salmonella* sp.

Medical laboratory science cut-up - the UK experience

Gordon McNair, Antrim Area Hospital, UK

Biomedical scientist (BMS) participation in examination and dissection (cut-up) of surgical pathology specimens has a chequered history in the UK. Pathologist support has been diverse over the past 20 years, with a tendency to polarise opinion, especially around BMS input into the more complex specimens. In the USA, the role of the advanced non-medical "pathologist assistant" is well developed.

A report published by a joint working party of the RCPath and Institute of Biomedical Science (IBMS) in January 2004 outlined a review of the experience of UK sites piloting the extended role of the BMS. It demonstrated that when principles of good practice are adhered to, standard operating procedures, training and audit measures are in place there was no perceived detrimental effect on the overall standard of histopathological reporting, timeliness, or professional practice.

Benefits cited included;

- Release of consultant time for other professional activities
- Increased job satisfaction and career opportunities for BMS
- Development of team-work within the laboratory
- More flexible and efficient use of cut-up facilities

The RCPath/IBMS introduced a training logbook and dissection examination process to cover specimen Categories B and C. As a result in recent years many departments have renewed efforts to develop BMS cut-up to varying levels. Over 30 BMSs have sat the Diploma of Extended Practice in Histological Dissection. Pathology departments throughout the country have witnessed many of the benefits cited above.

Solving the mystery sarcoma: a case study

Sarah Aikenhead, LabPlus, Auckland

A 24 year old female patient presented with a rapidly growing soft tissue tumour. This case was referred to our laboratory for review and further investigation. Various methods were employed to achieve the final diagnosis. These involved a cytological evaluation of the FNA mass, an immunocytochemical analysis of the cell block and finally FISH technique.

The cytology FNA Result showed "small round/oval blue cells" found in small clusters surrounded by slender capillaries. This finding, together with preliminary immunocytochemistry, revealed it could be a sarcoma suggesting the final diagnosis of a well-differentiated neuro-endocrine tumour. This rare diagnosis could only be confirmed by FISH.

Endometrial cells - good and bad

Reena Ramsaroop, Diagnostic Medlab, Auckland

The Cytology Department of Diagnostic Medlab serves the greater Auckland area with a population of 1.4 million. The total number of PAP smears processed per year is approximately 150,000. In September 2009 the National Screening Unit implemented 100% liquid base preparation. In 2005 the laboratory adopted the new Bethesda classification and recommendations for glandular lesions. This meant that all women over 40 with endometrial cells in their smears were documented (as compared to >50 in previous years).

We assessed the outcome in these women. A total of 889 women had endometrial cells in the smear; 805 benign endometrial cells and 84 atypical. Benign endometrial cells were documented in 614 women, <50 years and 191 women >50 years.

Hyperhomocysteinemia - a potential risk factor for osteoporosis

Dr Marcus Herrmann, NSW, Australia

Recent epidemiologic data suggest hyperhomocysteinemia as a new risk factor for fractures and low bone mineral density (BMD). In humans, circulating homocysteine also correlates with bone turnover and in particular bone resorption markers. Experimental hyperhomocysteinemia in rodents has been shown to reduce biomechanical bone strength (up to -40%) and cause substantial loss of cancellous bone (up to -90%). There is also evidence that homocysteine can accumulates in bone tissue via collagen binding. Cell culture experiments demonstrate that increasing concentrations of homocysteine strongly stimulates osteoclasts activity but have little effect on the bone forming activity of osteoblasts. Furthermore, there is increasing evidence that hyperhomocysteinemia interferes with collagen crosslinking, which is essential for mechanical bone strength.

Since hyperhomocysteinemia in adults is mainly caused by deficiencies of folate, vitamin B_{12} and B_6, cell culture studies were undertaken to investigate if decreasing concentrations of these vitamins affect the activity of cultured human osteoclasts and osteoblasts. These experiments revealed a strong stimulation of osteoclasts by decreasing concentrations of folate, vitamin B_{12} and B_6, which is accompanied by an accumulation of homocysteine in the culture medium. In contrast, human osteoblast activity was not affected. Human and animal studies investigating the role of folate, vitamin B_{12} and B_6 on bone metabolism have however provided conflicting results.

In conclusion, hyperhomocysteinemia adversely affects bone by an imbalance between osteoblasts and osteoclasts as well as an inhibition of extracellular collagen crosslinking. The role of B-vitamins in bone health is still insufficiently understood.

Nutritional management of inborn errors of metabolism - treating enzyme deficiencies with diet

Rhonda Akroyd, Auckland City Hospital

The New Zealand national newborn screening programme screens for 28 inborn errors of metabolism (IEM). IEM are individually rare, genetic conditions, but collectively the combined incidence is approximately 1:1100 births. These small molecule disorders result in a progressive accumulation of metabolites which are toxic to the newborn because a key enzyme in a metabolic pathway is missing. Such disorders include: aminoacidopathies, urea cycle disorders and organic acidaemias and those disorders involving energy metabolism; fatty acid oxidation disorders and glycogen storage disease. Diet management is the major treatment in many IEM.

Infants with an IEM appear normal at birth but subsequently deteriorate. Early diagnosis and commencement of treatment in the neonatal period is critical if serious morbidity and mortality are to be prevented. Many of the IEM require substrate restriction, product replacement, removal of toxic metabolites or pharmacological supplementation of co-factors or a combination of treatments. There are basic dietetic principles applicable to all conditions such as maintaining metabolic stability during times of rapid growth and implementation of appropriate nutrition support (including emergency regimens) during times of poor intake e.g. acute metabolic crisis. Pregnancy and breast feeding present particular challenges in IEM.

Acute treatment and long term management of many IEM are complex and require dietetic vigilance. Routine measures of biochemical markers such as phenylalanine levels in phenylketonuria and branched-chain amino acids in maple syrup urine disease are important considerations in the diet assessment and treatment. A close working relationship between dietician and laboratory services is critical in the management of many IEM.

Identification of Lancefield Groups A, B, D, F and G and *Streptococcus pneumoniae* by direct streptococcal grouping from Bactec blood cultures

Gavin Cooper, LabPlus, Auckland

The standard method for identifying Streptococci from blood cultures is immunological testing of an isolate growing on a solid medium. The feasibility of testing an organism directly from blood cultures has not been widely researched. This presentation describes the preliminary results of an ongoing prospective trial to identify Lancefield Groups A, B, D, F and G and *S. pneumoniae* directly from unspun Bactec blood culture media using the Prolex Streptococcal Grouping Latex kit and/or the Binax NOW *S. pneumoniae* kit.

The blood culture gram stain is assessed for Streptococci in chains or pairs. The direct streptococcal grouping is performed on un-spun blood culture media. Streptococci in chains are tested using the Prolex Streptococcal Grouping Latex kit only. Streptococci in pairs are tested using the Prolex Streptococcal Grouping kit and then the *S. pneumoniae* Binax NOW if the Prolex kit was negative.

The results from the first five months of the trial indicate that direct streptococcal grouping has a high positive predictive value. However, there have been a number of false negative results with Lancefield groups A, B and D. The trial will continue until a representative number of each *Streptococcal sp.* has been tested.

Clostridium difficile: taking the inconclusive out of *Clostridium difficile* testing

Terri Swager, LabPlus, Auckland

The increasing numbers and severity of Clostridium difficile disease globally has led us to look at which laboratory testing strategies are best to assist in diagnosis of this disease. Commonly EIA tests are used to identify the presence of C. difficile toxins A or B but currently available kits lack sensitivity.

Recently there has been recognition that screening for Glutamate dehydrogenase (GDH) is more sensitive for detecting the presence of *C. difficile* antigen. Although sensitive, GDH testing lacks specificity and fails to distinguish between toxigenic and non-toxigenic strains of *C. difficile*. Techlab TOX A/B Quik Chek Complete is a rapid reproducible assay which tests for both GDH antigen and Toxin A and B. This has provided a reliable screening test for *C. difficile* but a confirmatory test is needed when a sample tests positive for GDH but is Toxin A and B negative.

LabPLUS has evaluated GeneXpert *C. difficile* PCR assay that detects sequences in the genes for Toxin B, Binary Toxin and a mutation that has been shown to cause increased toxin production in "hypervirulent" and fluoroquinolone-resistant isolates. By using the two step approach to *C. difficile* testing, we have been able provide a rapid reliable screen test for *C. difficile* antigen and toxins, and subsequently distinguish between toxigenic and non-toxigenic strains in those samples that gave inconclusive results.

Laboratory errors and root cause analysis

Imelda Bromilow, Laterol Griffols

The purpose of pre-transfusion testing is to ensure that the right component of blood is selected and administered to the right patient. Laboratory errors can occur from the point of the tests being ordered to the final reporting of the results and therefore include errors of interpretation and technical or clerical problems. These can result in the wrong blood being issued and/or transfused provoking major morbidity. Other errors may involve handling or storage, or be associated with different aspects of pre-transfusion testing. For example, the correct sample may be selected and tested but incorrect results recorded. Procedural errors may involve the incorrect test selection, resulting in wrong, inadequate or inappropriate information being recorded.

Some errors can be due to lack of training or experience. For example, the ability to recognise mixed-field (double cell populations) reactions. Manual techniques are more prone to errors than automated procedures. However, training remains imperative for understanding the equipment and software to be able to utilise the technology most effectively.

Most errors can be avoided by initiating Total Quality Management, including the use of Root Cause Analysis techniques. What happened? How? Why? What are the consequences? When the reason for the error has been established, then corrective and preventative action should be implemented: What should be changed? How? When? By whom? Safeguards to defend against possible error-prone acts or omissions make each procedure as fool-proof as possible, aiming for zero errors and safe transfusion.

Routine antenatal anti-D prophylaxis : case study

Carole Watson, Blood Bank, Middlemore Hospital, Auckland

Counties Manukau deliver approximately 8000 babies a year. All should have cord samples taken. Middlemore Blood Bank routinely performs a group and DAT on those where the mother has an antibody, all Rh (D) negative mothers, and babies who develop jaundice.

On 11th April 2010 Mrs K, group A Rh (D) negative, antibody screen negative, delivered a normal healthy boy. The group of the baby was A Rh(D) positive and the DAT was positive. Anti D was eluted from the red cells. Mrs K had two doses of Anti D during the pregnancy. We have over 150 years of blood banking experience at Middlemore and from a diverse ethnic background, not one of us had come across prophylactic anti- D crossing the placenta. However a call to a TMS and some web searching, found that world wide it was 2-7% with one dose of anti- D, and can be up to 20% if two doses are given.

A quiet night with Kawasaki

Christian Christian, Southern Community Laboratories, Invercargill

Kawasaki disease is an acute febrile illness in infancy and childhood. It was first described by Professor Tomisaku Kawasaki in 1967. It is considered to be an autoimmune disease and mainly characterized by inflammation of the blood vessels, mucous membranes and lymph nodes. A higher incidence is found in male children of Asian descent aged between 18 -24 months. It is the leading cause of heart disease in children. The main symptom is fever in association with four of the following five criteria; rash, conjunctivitis, redness and swelling of the lips, throat and tongue, non-purulent cervical lymphadenopathy and redness and swelling of the hands and feet. Complications such as aneurism, myocarditis and valvulitis may occur due to the weakening of the heart. Together with its symptoms, it can be diagnosed by looking at the laboratory findings as well as the medical imaging results. The main treatment for Kawasaki disease is Intragam, which should be given within 10 days of the onset of fever.

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Is the 30 minute rule still appropriate?

Holly Perry, Auckland University of Technology

Purpose: The 30-minute rule, whereby intact red cell products may be returned to stock if returned to 4°C storage within 30 minutes of issue, was established many years ago. It was based on observations that units of whole blood, removed from storage temperature of 1°C- 6°C, and left at room temperature, would reach a core temperature of ≥10°C between 45 minutes and 1 hour.

Methods:

Part 1 (2008)

41 red cells resuspended leucocyte depleted (RCRLD)and 8 paediatric red cells resuspended leucocyte depleted (PRCRLD) were subjected to exposure to ambient temperature for time intervals between one and sixty minutes. Core temperatures of all units were measured at one minute intervals.

Part 2 (2010)

Safe-T-Vue temperature indicator devices were applied to 30 pre-cooled RCRLD. Temprecord monitors were inserted and measured temperature every 30 seconds. Three environmental situations were tested (hanging on filtration rack, resting on a metal and on a formica bench).

Results:

Part 1

RCRLD units reached an average core temperature of 10°C at 15 minutes, 12.7°C at 30 minutes, and 15°C at 60 minutes. PRCLD reached an average core temperature of 12.8°C at 15 minutes, 15.5°C at 30 minutes, and 17.8°C at 60 minutes.

Part 2

Colour change on the Safe-T-Vue units hanging on the filtration rack occurred at 30 minutes, indicating the units had reached an average core temperature of 10°C. Results were combined for blood bags on the metal and formica-type benches. Units reached an average core temperature of 12°C at 17 minutes.

Conclusions: The 30 minute rule is appropriate under some conditions, but not under others. Factors in an environment such as air flow, ambient temperature and space could determine the amount of time that units will take to determine 10ºC. Implications for patients and blood services are under consideration.

UK experience with the introduction of LBC and HPV triage testing

Nick Dudding, Sheffield Teaching Hospitals, East Pennine Cytology Training Centre UK

Liquid based Cytology (LBC) was recommended for introduction into England in October 2003, largely because of our dreadful unsatisfactory / inadequate rates. Plans for its rollout followed fairly quickly after that, but because of the sheer scale of the process, with all sample takers and laboratory staff having to be retrained, a small number of laboratories did not complete the process until October 2008.

In England responsibility for strategic direction and commissioning of this process was given to the 28 Strategic Health Authorities (SHAs). Complicated English governmental organisations that I won't cover in any detail. The first task was to decide which of the two available LBC systems were to be used. In my local area it was decided that based on potential cost savings from joint commissioning all laboratories would use the same system. Following a comprehensive scoring process, which incorporated everything from sample taking, transport, processing through to the actual consumable and staffing costs, SurePath™ won the contract. The initial contracts ran for five years with the result that some laboratories in England are just about to enter a second period of negotiation. With a cost difference in favour of SurePath™ of around $2 per sample and four million samples per annum taken in the publicly funded English programme one might have thought that SurePath™ would have been even more successful, but concerns over chain of custody, the amount of preparation work and more bizarrely the slightly different staining meant that each company ended up with around a 50% share. Hologic® was most successful in the South of the country with SurePath™ achieving greater success in the North.

The biggest immediate impact on laboratories was the decision that not all would be allocated a processing machine. Based on local geography and optimum workloads many "hub and spoke" re-organisations took place, with one laboratory processing samples, but screening staff remaining where they were. This model has proved largely successful and allowed many smaller laboratories to continue to operate and staff to remain in employment.

In terms of sensitivity / specificity and the likely impact on the incidence and mortality of cervical cancer there is little, if any, evidence to say that LBC impacted one way or another. Shortly after the introduction of LBC the government took the decision to change screening intervals from five to three years for 25 - 50 year olds and removed screening completely from those under 25. This has meant that before and after comparisons, always difficult, have become impossible. What clearly have improved however are the unsatisfactory or inadequate rates. Prior to LBC the English mean was 10%, this has fallen to 3% across the entire country and in Sheffield, where we use Surepath™ just 1%. If you couple this with an increase in productivity of around 20 - 30% then the other age old UK problem of the screening backlog has also started to disappear. I doubt this would be included as a benefit in countries like Australia, but in the UK it was not unusual for women to have to wait for well over four weeks to receive their test result.

The latest data (http://www.ic.nhs.uk/webfiles/publications/cervicscrneng2008/Cervical%20Screening%20Bulletin%2007-08.pdf) shows that the percentage of tests reported within four weeks has risen to an all time high of 83%.

Whilst we cannot prove that LBC has impacted positively on disease detection it is clear that sample quality, as indicated by the presence of transformation zone (TZ) cells has improved. Within Sheffield teaching Hospitals we now find that 93% of samples contain TZ cells, a figure matched by that in Manchester (1). Whether this has impacted on overall dyskaryosis rates can be argued, but a recent audit in the North East, Yorkshire & Humber region has shown that it is almost certainly enhanced the pick up of endocervical lesions. The number of cases reported rose from a rate of 2.19 per 10,000 samples to 3.95. This increase of 80% occurred at the same time as positive predictive values (PPV) for samples coded as showing endocervical abnormality with a final histological outcome CGIN or invasive endocervical adenocarcinoma increased from 70.4% to 74.4%. More impressively, PPV for an outcome of any high grade disease increased from 92.6% to 95.3%. It is also worth reporting that the number of cases reported as showing borderline glandular change fell by 34% (2)

HPV testing

One of the major other advantages of introducing LBC is the potential to introduce adjunctive tests, in particular HPV testing. In England we have seen the beginnings of the introduction of test of cure (ToC) and triage of low grade changes.

HPV triage undertaken at the time of the LBC pilots in 2001 had indicated that:

- HPV triage was feasible
- Acceptable to women
- Accelerates the diagnosis of high-grade CIN
- Avoids the need for repeated cytology
- Could be cost effective in terms of quality and of life years saved(3, 4)

In order to assess this potential six English Sentinel sites for HPV triage and ToC commenced testing in Spring 2008. The full Triage protocol is complicated, but in essence women with a first borderline or mild dyskaryosis (i.e. following routine call or recall), have their sample sent for HPV testing and the cytology result is not reported until the HPV result is available. A HPV positive result is recorded on the basis of 2.0 RLUs using the Hybrid Capture 2 (HC2) test http://www1.qiagen.com/ which tests for 13 of the most common high risk types.

A borderline or mildly dyskaryotic cytology report with a positive HPV test results in referral to colposcopy. A HPV negative result leads to the cytology report being over-ridden and the women remain on normal (3/5 years) recall. At colposcopy women with high grade CIN are treated, those with no CIN on biopsy or a negative colposcopy are returned to normal recall and those with CIN I or a Colposcopic appearance of high grade are invited back for an early repeat (six or 12 months depending on whether the CIN I was treated or not). Interestingly, because not all koilocytic samples will test positive for high risk HPV types laboratories are "banned" from reporting HPV in the cytology report to avoid any complications of a cytology report stating HPV is present and a HC II result of negative.

The same sentinel sites are also taking the opportunity to investigate the potential of HPV testing following treatment for CIN. Again using Hybrid Capture II the post-treatment protocol in the sentinel sites comprises:

Cytology at six months

- If cytology is abnormal, refer for colposcopy
- If cytology is normal perform an HPV test
- If HPV positive refer for colposcopy
- If HPV & cytology negative, return to normal recall.

The results of both triage and ToC in this first year are currently being reviewed by the steering group, but despite initial reservations I can confess to being a strong advocate of both.

Whilst there is an obvious challenge to cytology in that we don't have the final say on management the benefits to women are undeniable. It:

- Speeds up referral
- Reduces number of re-tests
- Returns women to normal recall earlier

- Avoids referral for those that don't need it

Because of the psychological issues associated with testing for a STD one of the most vital aspects of the introduction of HPV testing were the educational sessions for sample takers. In Sheffield we ran a number of evening workshops to which all sample takers were invited. We ran through the protocols and discussed the role of HPV in the aetiology of cervical cancer. These proved invaluable and we were able to put to bed many myths surrounding HPV and give clinicians the background information to ensure they were able to answer any questions women might have. This lead to a very smooth and therefore successful introduction of triage and to my knowledge, although women are given the option to opt out of HPV testing, virtually none do.

As I write this abstract full results are unfortunately not available. However, early results in Sheffield show that 67% of those cases reported as borderline and 88% of those reported as mild dyskaryosis tested positive. What this all means needs further investigation since what is critical is the percentage of women that actually have high grade CIN. Early data suggests this might be in the region of 20 -25%, while a further 50% might have no disease at all. What this tells me is that medical economists might be needed.

Clearly, as found in the ALTs(5) trial (http://jnci.oxfordjournals.org/cgi/content/full/93/4/293) we shall have to debate the value and cost effectiveness of triage on mild dyskaryosis, but I expect this to be given the green light because whilst very high rates were found in Sheffield, this is not the case in all of the laboratories, and in some a significant number of women were "saved" from a visit to colposcopy. Certainly there are some very interesting early indications that there are significant inter-laboratory variations in HPV positivity and so the value of HPV testing will vary from laboratory to laboratory, not to mention region to region!

With regards ToC, the results seem even more promising. In Sheffield only 13% of women tested positive. In other words 87% of women were returned to normal recall, where previously they would have half annual cytology for 10 years. Although it will be a few years before we can be sure of the safety of this policy this is clearly one area where medical economists are not needed.

Consideration will have to be given to the known risks of a false negative HPV test. Despite the hype surrounding HPV testing and HCII in particular we know there is a risk of false negative results, not least if the vial is acellular. Jones et al(6) reported that only 83% of CIN IIIs in their series were HPV positive by HCII, whilst in the ARTISTIC trial (7) 31 out of 450 cases of CIN or III in the revealed group were negative by HCII.

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Drug screening by LCMS/MS. A major operational change

Sharon Paterson, Toxicology, Canterbury Health Laboratories, Christchurch

Purpose: Urine drug screening has been carried out in the Toxicology Laboratory at Christchurch Hospital for the last four decades using a combination of thin layer chromatography, immunoassay and spot tests for specific compound classes. More recently GCMS screening has been added as an adjunct. An LCMS drug screening system has recently become available with the added bonus of a standardised library that allows routine drug screening to be performed without the need for operators to build their own LCMS specific library.

Method: An evaluation has been performed comparing the Applied Biosystems CLiquid drug screening protocol on an ABI 3200 QTrap LCMSMS system with the incumbent TLC system.

Results: Over 1000 samples were directly compared between the two systems with 85% giving equivalent results and the remaining 15% revealing extra compounds detected by the LCMS system.

Conclusion: The LCMS drug screening process has been incorporated into the routine analyses performed in the toxicology laboratory with minimal disruption.

The role of rapid diagnostic techniques in diagnosis and susceptibility testing of TB

Dr Joshua Freeman, LabPlus, Auckland

Active TB infections due to multi-drug resistant TB (MDR-TB) and extensively drug resistant TB (XDR-TB) are becoming more common worldwide and in New Zealand. Compared to infection with susceptible TB, MDR-TB and XDR-TB are associated with poorer outcomes, a greater risk of drug toxicity and greater expense to the health system. Of particular concern, few new anti-TB drugs have come to the market during the last 30 years and it is unlikely that new drugs will become available in the near future. It is therefore imperative that aggressive measures are taken to minimise the risk of further resistance developing to anti-TB drugs in New Zealand and worldwide. Delays in the laboratory diagnosis of TB and/or delays in the diagnosis of MDR-TB/XDR-TB status lead to inadequate treatment and further resistance. Mycobacteriology laboratories in New Zealand must work hard to reduce turnaround times on TB culture and drug susceptibility testing for TB. Rapid diagnostic techniques using nucleic-acid amplification tests such as the Cepheid GeneXpert® and the Hains line probe® are able to detect TB and MDR-TB genotype directly on clinical specimens. Such commercial assays are likely to play an important role in TB control in New Zealand by reducing the time to diagnosis of MDR-TB, thus reducing the risk of inadequate treatment regimens and resistance development.

Genetic diagnosis of pregnancy tissue - an overview of testing and its impact on families

Sheridan Daly, LabPlus, Auckland

Genetic diagnosis of pregnancy tissue is an often overlooked area, yet loss of a pregnancy is something that is likely to affect almost every family at some stage. In some cases testing reveals inherited aberrations which can help explain recurrent loss and also have implications in the future planning and management of pregnancies. In other cases, age-related or random abnormalities are detected which do not indicate an increased recurrence risk. In these cases a result can help assuage the guilt associated with a termination or loss, and reassure families who want to try for another pregnancy. As a diagnostic laboratory it is easy to view samples from a purely scientific point and neglect the personal relevance. However, it is important to consider and appreciate the value of this testing and the impact that the diagnosis, even of a simple trisomy, can have on a patient and their family.

Going round in circles! Prenatal detection of a ring chromosome 2

Roberto Mazzaschi, Diagnostic Genetics, LabPlus, Auckland

Ring chromosomes are uncommon, and when they do occur, they almost always arise sporadically. Their formation involves loss of genetic material at the points where the chromosome ends (telomeres) fuse. They are therefore associated with a varying degree of phenotypic abnormality. We report a case of non-mosaic ring chromosome 21, detected through routine karyotyping of an amniotic fluid sample. Parental karyotyping showed that the ring had been inherited from the mother, a mosaic carrier. Surprisingly, the father also had a chromosome rearrangement unrelated to the ring 21. Molecular karyotyping was then undertaken on the amniotic fluid sample to characterise the deleted region. Following detailed ultrasound scanning and genetic counselling, together with a review of the family history, the couple decided to continue with the pregnancy. The baby has since been born. This example illustrates how different medical and scientific fields are brought together to resolve difficult cases.

The use of tetra-primer amplification refractory mutation system (ARMS) PCR in the diagnostic setting

Renate Marquis-Nicholson, Molecular Genetics Laboratory, LabPlus, Auckland

Purpose: With the increasing integration of molecular genetic analysis into routine clinical practice, the demand for rapid, bespoke testing is growing. It is important for diagnostic laboratories to be able to meet this need with a simple, cost-effective and high-throughput technique.

Methods: We designed primers to allow tetra-primer ARMS PCR analysis of a range of samples in a number of clinical settings. These included:

1) confirmation of a specific point mutation in the context of elevated citrulline levels detected on newborn screening. This particular mutation leads to a benign clinical course which requires observation, rather than active treatment
2) predictive testing for a familial mutation responsible for non-autoimmune hyperthyroidism
3) SNP detection as part of a clinical trial of a novel functional therapy following cerebrovascular accident

The accuracy of each assay was confirmed via conventional PCR and bidirectional sequencing.

Results: Tetra-primer ARMS PCR was able to detect the variant of interest in each of these settings, with a turn-around time of approximately 6 hours. It was effectively applied to DNA extracted from blood and from Guthrie card spots.

Conclusions: Tetra-primer ARMS PCR enables the rapid and accurate detection of single nucleotide variants. Using the online tool freely available at http://cedar.genetics.soton.ac.uk/public_html/primer1.html, primer design is simple and easily tailored to the variant of interest. The assay is economical and a definitive answer can be given within 6 hours of initiating testing. Tetra-primer ARMS PCR is therefore, a useful tool in the diagnostic setting where accuracy, flexibility and cost-effectiveness are key concerns.

The importance of H&E and Elastic stain

Sonya Prasad, Martyn Peck, RCPA, Australia

Laboratories in Australia must be accredited to be functional and one ISO 15189 requirement is for labs to be enrolled in an external quality assurance program. The H&E stain is the most commonly used stain for histological diagnosis and the use of a elastic stain is important for identifying elastic fibres which is useful to identify changes in the elastic laminae of blood vessels that may occur with hypertension, or list with degenerative process. Elastic fibres may be increased due to a stromal reaction to the presence of tumour cells, as in breast cancer, or secretion by tumour cells e.g. elastoma dorsi. The elastic staining and H&E exercise results is dependent on appropriate handling, fixation and processing of tissue samples. Unstained sections were provided to participants for preparation in their laboratories with these stains. The submitted slides were then reviewed by a committee of their peers and assessed for technical quality.

Pathology in the UK

Dr Stephen Absalom, LabPlus, Auckland

From the perspective of an involvement in NHS pathology stretching over 36 years, in many different laboratories changes have taken place. The pace of change has accelerated in recent years creating increasing uncertainty amongst pathology staff. The basic building block of the NHS has for a long time been the District General Hospital providing secondary care services to a defined community but also hosting many services which also support primary care such as pathology. The organisation of pathology services has therefore inevitably been linked to the organisation of hospital services and it is this linkage which current changes seem likely to break. The UK model for pathology is of a medically led clinical service but the reforms now driven by financial pressures threaten this.

Clinical scientists - expanding role

Chris Kendrick, Massey University, Palmerston North

Future Workforce (FW) is the District Health Boards New Zealand (DHBNZ) strategic plan to progress the goal of sector wide development of the health and disability workforce. It focuses on DHB's collective priorities and actions for health and disability workforce over the next 5-10 years. In 2006 the FW established a Think Tank group to progress work toward the development of an extended role for MLS in this country. The TT was to consider the development of the Clinical Scientist in response to anticipated future shortages of key laboratory personnel and the introduction of more complex future technologies. The development of the Clinical Scientist role in the UK has lead to Scientist membership of the RCP and full clinical consultant status. NZ faces similar laboratory workplace issues to those in the UK and the Clinical Scientist has been identified as a way to allow NZ laboratories to meet the technological challenges ahead.

The mystery of unclassified genetic variants: what they are and how we interpret them

Clare Brookes, Molecular Genetics, LabPlus, Auckland

Purpose: DNA alterations can be classified into two distinct groups - polymorphisms and mutations. Polymorphisms are benign changes in contrast to mutations which are disease-causing. The challenge arises when a DNA variant is identified which falls into neither of these two groups. These are known as unclassified variants and are DNA alterations with an unknown function. The interpretation of these unclassified variants is complex yet essential for providing clinicians with comprehensive patient results. The purpose of this presentation is to discuss the process of identifying unclassified variants and then determining the pathogenic relevance of these.

Methods: Upon identification of a genetic alteration our initial task is to confirm if the change is a known polymorphism or a disease-associated mutation through the use of specific diagnostic databases. If the alteration is neither, we describe this as an unclassified variant and determine its pathogenic significance using a selection of online programs; namely Splice Site Prediction, PolyPhen-2 and SIFT.

Results: We have interpreted unclassified variants with varying degrees of pathogenic significance.

Conclusions: This study highlights our process of interpreting the pathogenic significance of unclassified genetic variants and how this is of great importance in managing patients referred for genetic testing.

Automation in the molecular genetics laboratory

Jamie-Lee Day, Molecular Haematology, LabPlus, Auckland

For over 30 years, automation in haematology and biochemistry has become common- place in medical laboratories. Automation of the genetic diagnostic sections has been slow to catch up and is only now beginning to be accepted for a limited number of high throughput tests such as Haemochromatosis HFE gene mutation analysis. The bottle-neck has often been the initial extraction of DNA but there are now numerous platforms which have automated this step and downstream processing is now also being automated with a variety of PCR-based assays set-up by robots and SNP analysis performed in Real-Time instruments. Refinement of these steps has meant that real savings can be made in the laboratory as new instruments arrive on the market and technologies are updated.

Genetic profiling: implications for diagnosis, treatment and monitoring of acute myeloid leukaemia

Nikhil Singh, LabPlus, Auckland

Recent advances in the genetic analysis of acute myeloid leukaemia (AML) have had a profound effect on the management and treatment of patients. Genetic testing is now an integral part of the diagnosis, treatment determination and monitoring of residual disease. The heterogeneity of AML has lead international groups such as the WHO and the UK-based MRC group to stratify patients into favourable, intermediate and adverse prognostic groups. Although cytogenetic analysis remains a critical factor in diagnosis and prognosis, over 40% of AML patients have a normal karyotype. Further refinement in the management of these patients has been provided with the introduction of biological markers such as FLT3, NPM1 and CEBPA. Newer markers are also emerging which will influence the treatment and prognosis of this heterogeneous group.

A miscellany of challenging presentations in liquid-based cervical cytology

Stuart Dobson, Hologic Inc

Below are discussed the cytomorphologic criteria and differential diagnoses of five challenging presentations encountered in liquid-based cervical cytology specimens.

Follicular cervicitis

The presence of an admixture of lymphocytes of varying range of maturation accompanied by tingible body macrophages may indicate a chronic inflammatory condition or the rupturing of a lymph follicle during sample collection. On the ThinPrep slide the lymphocytes from chronic follicular cervicitis will generally appear in small clusters grouped together. Compare that with the characteristic streak of lymphoid cells more often noted on the conventional Pap smear. The presence of tingible body macrophages aid in the diagnosis. Possible differential diagnoses of follicular cervicitis include high-grade squamous intraepithelial neoplasia (HSIL), small cell carcinoma, and endometrial adenocarcinoma. While single lymphoid cells may appear to cluster together on liquid-based cytology there will be no evidence of syncitia, sheets, or gland formation that might be observed with epithelial neoplasia. Mature lymphocytes predominate in follicular cervicitis patterns and would typically be smaller than the cells associated with the entities described above as potential differential diagnoses. With specific reference to HSIL, invariably a spectrum of change is noted that would include lower grade dysplastic change. Follicular cervicitis would lack this spectrum of cellular change.

Another possible differential diagnosis with follicular cervicitis, albeit rare, is malignant lymphoma of the cervix. A range of maturation within lymphoid cells associated with follicular cervicitis. In contrast, malignant lymphoma would typically appear as a monotonous population of pleomorphic lymphoid cells of similar maturation and typically lacking tingible body macrophages (except when high-grade).

Decidual cells

Decidualisation is a transformation of the endometrial stroma during pregnancy. Hormonal stimulation can induce decidualisation of stromal cells of other sites than endometrium. Nodules of decidualisation may form in the cervical stroma, but decidual cells appear in the smear in case of abrasion of the surface epithelium or decidual polyp. It can cause vaginal bleedings and even mimic a carcinoma at colposcopy. Decidual cells have an abundant, polygonal, pale, eosinophilic or basophilic cytoplasm with heavier cell outlines than squamous metaplasia. Their nuclei are slightly enlarged, sometimes prominently nucleolated, round or oval with bland vesicular chromatin. The morphologic characteristics of decidual change on ThinPrep Pap test slides are similar to those found on the conventional Pap smear.

They can easily be misdiagnosed as condyloma or low-grade squamous intraepithelial lesion (LSIL) or even HSIL, especially when the nuclear staining is too dark or poorly fixed. LSIL will display coarser chromatin and lack nucleoli than decidual change. Another differential diagnosis is repair which invariably presents in larger sheets and rarely as single cells. In contrast, decidual cells present as single cells or small clusters.

Radiation change

Radiation treatment of malignant lesions in the female genital tract traumatizes the epithelium causing ulceration that can cytologically mimic dysplasia or carcinoma. The extent of this trauma depends on the type and duration of therapy; therefore the cellular changes may resolve within six months (acute radiation change) or can persist for a lifetime (chronic radiation change). Some chemotherapeutic agents can also produce similar cellular changes, making patient history a valuable tool. Post-radiation dysplasia (PRD) has been reported in up to 23% of patients with the atypia resembling that of non-radiated patients. Considering the difficulties in distinguishing PRD from recurrent carcinoma, colposcopy and biopsy are recommended to rule out the latter.

Cytomegaly is the most well known radiation related change.

Cytomegalic cells have enlarged and/or multiple nuclei and abundant cytoplasm while maintaining a fairly normal N/C ratio. The nuclei can be pale and the chromatin can be finely granular to "smudgy" with possible micro- and macronucleoli and vacuoles. The cytoplasm may exhibit **bichromasia** and **vacuoles** with or without engulfed polymorphs. Bizarre cell shapes may also be seen. The background of these specimens will contain degenerated blood that appears "stringy", cellular debris, polymorphs and histiocytes. **Reparative changes** can be seen frequently and occur in single-layered, cohesive, streaming sheets with pale nuclei and prominent nucleoli. Radiated fibroblasts are present and have delicate cytoplasm with vacuoles and cytoplasmic projections. The nuclei are pale, finely granular with possible nucleoli. Degenerated tumour cells may be seen in specimens initially following radiation.

Acute radiation change, as described above, typically presents for 6-8 weeks after administration of radiation therapy. Following this degenerated and viable appearing tumour cells are expected to clear. The absence of an acute response to the radiation therapy at around -8 weeks is deemed to be a poor prognostic factor. Chronic radiation change can persist for years with similar cytomorphologic features as noted in acute radiation change but usually lacking the reparative change and inflammatory response.

Distinguishing radiation changes in malignant cells from radiated benign cells may present challenges in liquid based cytology similar to those encountered on conventional Pap smears. A key morphologic criterion will be the N:C ratio which will appear relatively normal in benign cells despite cytomegalic changes. Radiated malignant cells will generally display higher N:C ratios, courser chromatin, and greater hyperchromasia. Following an 8-week period after radiation therapy the presence of mitotic figures in radiated malignant cells is indicative of persistent viable tumour. Malignant cells without obvious radiation change noted cytologically at this point denote recurrent cancer.

Tubal metaplasia
Tubal metaplasia is characterised by columnar cells usually from the upper endocervix with mildly pleomorphic, hyperchromatic nuclei and increased N:C ratios. Nucleoli are absent or inconspicuous. Cytoplasm may appear dense and granular. As with conventional Pap smears, cells from tubal metaplasia may occasionally be mistaken for atypical endocervical cells and even adenocarcinoma in situ (AIS). In order to avoid an over-diagnosis, look for the presence of discrete cytoplasmic vacuoles, **terminal bars and cilia**.

The fluid-based fixation method also may help in accurately classifying other entities such as tubal metaplasia and cells of true lower uterine segment, AIS, and squamous lesions involving glands. The smearing technique employed with the conventional Pap smear can be harsh and destructive to cellular material, making the identification of cilia difficult. With the ThinPrep Pap Test, the rinsing technique and gentle collection and cell transfer utilized by the ThinPrep System enhances the preservation and ultimately the visualization of the fragile cilia which permits recognizing tubal metaplasia.

Tubal metaplasia can closely mimic AIS. However, on close inspection the abnormalities such as crowding, nuclear elongation, irregular nuclear membranes and abnormal tissue fragments are less severe. Locating cilia and/or terminal bars favours, if not, confirms benignancy.

Repair
The reparative response to mucosal damage caused by inflammation, delivery, ablative therapy, biopsy, or radiation can present some challenges in interpretation. In general, the cellular presentations seen in conventional Pap smears is similar to that noted in liquid-based cytology preparations. Yet repair does present one of the more alarming differential diagnoses encountered in cervical cytology – specifically atypical repair versus poorly differentiation squamous cell carcinoma (SCC).

Reparative cells present almost exclusively in cohesive sheets. The sheets often have a loose appearance with open space often noted between cells. The sheets are flat with polarity maintained; however one may note some rounding up of groups in liquid-based cytology preparations. N:C ratios of cells are relatively normal. In comparison, SCC will present as single cells and sheets with higher N:C ratio, usually lacking polarity and displaying multiple planes of nuclear focus

Variability in nuclear size is a feature of repair, and in particular atypical repair. However, there may be minimal variation in nuclear chromatin which should appear finely granular, evenly distributed, and normochomatic. Nuclear contours should be smooth despite the variation in nuclear size. On the other hand, SCC will display pleomorphism in all aspects of the nuclear appearance.

Nucleoli are prominent in reparative cells, often more prominent than noted in cancer. They may appear single or multiple and commonly all cells in a sheet may display nucleoli. There is typically greater variation in the presence, absence, and number of nucleoli seen in SCC.

Ingested polymorphs within the cytoplasm of reparative cells are a common feature that favours a diagnosis of repair. Mitotic figures may also be noted. The background of repair may reveal increased inflammation and possibly pathogen.

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Molecular epidemiology and susceptibility profiles of *Clostridium difficile* isolates in New Zealand, 2009

Nadia Al Anbuky, S Paviour, T Camp, T Swager, S Roberts, C Pope, H Heffernan. LabPlus, Auckland and ESR, Porirua

Little is known about the *Clostridium difficile* strains that are currently circulating in New Zealand or whether the epidemic hypervirulent strain, PCR ribotype 027, is present. Eight laboratories, from throughout New Zealand, participated in the survey. Faecal specimens that were *C. difficile* toxin positive were submitted for culture. Specimens were cultured on to CCF agar and isolates were identified by their colonial appearance and typical biochemical profile. Susceptibility testing was carried out using the agar dilution MIC method and, where available, CLSI interpretive criteria were applied. The antimicrobial agents tested were penicillin, piperacillin-tazobactam, vancomycin, ciprofloxacin, moxifloxacin, clindamycin, clarithromycin, meropenem and metronidazole. Isolates were PCR ribotyped according to the method used by the National Public Health Service for Wales.

Between 1st February and 2nd June 2009, 159 toxin-positive faecal specimens were cultured. 101 non-duplicate *C. difficile* isolates were obtained from 97 patients. Most isolates were fully susceptible to the range of antimicrobial agents tested. Isolated resistance to macrolides, clindamycin and fluoroquinolones was seen. 32 PCR ribotypes were identified among the 101 isolates.

The most common PCR ribotypes were 014 (18 isolates), 002 (11 isolates) and 005 (10 isolates).Three novel PCR ribotypes (295, 296 and 298) were identified.

There was a wide range of *C. difficile* ribotypes circulating in New Zealand. Some types were common to several patients and more than one geographic area. A cluster of patients with the same type was only evident in one area. The hypervirulent strain, PCR ribotype 027, is not present in New Zealand and antimicrobial resistance currently is uncommon.

The Neurological Foundation of New Zealand Human Brain Bank: collection and processing of human brain tissue for research

Frances Biggins, University of Auckland

Purpose: To understand more completely the neuroanatomy, neurochemistry and neuropathology of the normal and diseased human brain, it is essential to have access to optimally preserved post mortem human brain tissue.

Method: The Neurological Foundation of New Zealand Human Brain Bank, based at the University of Auckland, has an established protocol for the preservation of adult donor human brain tissue. Usually, the whole brain is perfused first with 1% sodium nitrite, followed by 15% formalin; immersed in 15% formalin for 12-24 hours; dissected into blocks according to a strict regional mapping plan; the blocks are fixed in formalin for a further 12-24 hours; transferred into 20% sucrose for one week, then 30% sucrose for 4 weeks; snap frozen with powdered dry ice; sealed in foil and stored in containers in a -800C chest freezer. Depending on the type of research being done, the two hemispheres may be separated, one for perfusion-fixation and the other remains unfixed and is snap frozen after dissection.

Results: Preparing and processing the tissue in this way allows investigative studies using several techniques – autoradiography, in situ hybridisation, DNA/RNA analysis and immunohistochemistry.

Conclusions: The success of the Human Brain Bank and its continuing expansion is due not only to the wide range of methodologies and national/international collaborations it allows in the study of the normal and diseased brain, but also, importantly, it is due to the close association with donor families and community groups involved with neurological disorders such as Alzheimer's disease, Huntington's disease, Parkinson's disease, motor neuron disease and schizophrenia.

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A 9q34.4 duplication derived from familial interchromosomal insertion – a case study using chromosome microarray assay and FISH

James Chang, LabPlus, Auckland

A 15 year old female presented with developmental delay and mild dysmorphic features, including retinal dystrophy. Chromosomal microarray analysis (CMA), on a peripheral blood specimen, showed the presence of a 2.1 Mb duplication at chromosome 9q34.4. Confirmation testing was done by fluorescence in situ hybridization (FISH). This showed the extra copy of the region in 9q34.4 detected, was located on a derivative 19 chromosome. No copy number imbalance for chromosome19 was detected in either the FISH or array analysis. Parental tests were then carried out to clarify the origin of this abnormality and provide chromosomal evidence on genetic counselling for possible future pregnancies.

Persistent weeping willy

Julie Creighton, Canterbury Health Laboratories, Christchurch

Purpose: Urethritis is a common complaint for men visiting the Christchurch Sexual Health Clinic. 9.4% (294/3133) of these men have *Chlamydia trachomatis,* 1-2% have *Neisseria gonorrhoeae.* However, there is a group of men with persistent or recurrent urethritis which is non-Chlamydia, non-gonorrhoea (NCNGU) and for whom management can be problematic. Recent studies have established *Mycoplasma genitalium* as a cause of urethritis in men and possibly cervicitis and PID in women. Limited studies have been performed in New Zealand. *Trichomonas vaginalis* was also investigated due to the difficulty of detecting this organism by conventional techniques.

Method: Predominantly first catch urines and some MSU's from selected patients were tested by an in-house PCR with *M.genitalium* and *T.vaginalis* probes.

Conclusions: The *M.genitalium* / *T.vaginalis* PCR test is a useful tool for the management of patients with NCNGU.

Comparison of erythrocyte sedimentation rate (ESR) measurement by the automated Mixrate-X20 analyzer and manual westergren method

Carmen Janssen-Sahebzad, Pathology Associates

Objective: To compare the performance of the Mixrate-X20, an automated analyzer for the measurement of erythrocyte sedimentation rate (ESR), with the manual Westergren (reference) method.

Materials and methods: Both methods were applied to 44 randomly selected patients. The linear regression including Pearson's regression analysis were used to measure the correlation. Bland and Altman data analysis methods were used to measure the agreement between the automated and manual method. A separate test was applied to 29 randomly selected patients. The F-test was used to assess the precision of test results. The coefficient of variation (CV) was calculated and the two sets of result were line charted for precision testing.

Results: The regression analysis showed a good correlation between the two methods (r = 0.99). The Bland and Altman data analysis showed no systematic bias (95% confidence interval for mean difference); however, limits of agreement were between 7.99 and -9.09. This indicates that ESR values measured by the Mixrate-X20 may be 7.99mm/h above or -9.09mm/h below the reference method. A greater scatter of data was also observed with abnormally high (>20mm/h) ESR results (mean of difference = -3.4 and limits of agreement = 6.1 and -12.9) compared with normal (<20mm/h) readings (mean of difference = 1.3 and limits of agreement = 6.4 and -3.9).

Conclusions: The Bland and Altman statistical analysis showed a wide degree of scatter between results obtained by the two ESR techniques that was not clearly demonstrated using the linear regression analysis. The automated Mixrate-X20 system tends to underestimate ESR values, but not to a clinically significant degree. The F-test proved that the Mixrate-X20 produces reliable results.

Specificity and sensitivity of Papanicolau test method in detecting cervical cancer precursors [CIN/SIL] and progression to invasive cancer

Manasa Mainaqelelevu, Fiji School of Medicine

Introduction: Cervical cancer, which develops from cervical intraepithelial neoplasia (CIN), is an important cause of death in women worldwide. The introduction of the Pap smear test a few decades ago has really reduced the incidence of cervical cancer in most developed and some developing countries as well. However, concern about the low sensitivity of the Pap smear test in detecting cervical cancer lesions has prompted a search for newer methods to either supplement or replace it.

Methodology: A comprehensive internet-based search, using combined mesh, thesaurus and general searches was conducted on the sensitivity and specificity of Pap smear (conventional cytology) test method in detecting human papillomavirus and its progression to invasive cancer in comparison to other common cervical cancer screening methods. Any study and review available were reviewed.

Results: Using the inclusion and exclusion criteria, twenty three (23) articles were selected of the 30 articles found for the topic discussing common screening methods for cervical cancer and their sensitivity and specificity in primary screening of cervical cancer. Of the 23 articles, 10 were randomised trial, 9 cohort study, 3 cross-sectional, and 1 economic evaluation article. 15 articles on Pap smear vs. HPV DNA test, 3 articles on Pap smear vs. LBC, 1 article on visual vs. Pap smear, and 4 articles on Pap smear vs. two other methods.

Discussion: HPV-DNA testing method which is expensive is the most sensitive test to detect cervical cancer precursors followed by the liquid-based cytology compared to the Pap smear method. However, both the HPV-DNA test and the liquid-based cytology have low specificity than the Pap smear. HPV DNA is more sensitive among younger women (30 years or less) and less sensitive on older population compared to LBC and Pap smear. Both the latter methods are sensitive on older population. HPV DNA lengthens the screening intervals compared to other methods and also increases the number of referral for colposcopy with LBC. In terms of cost, HPV-DNA is expensive compared to other methods but saves slightly more lives.

Conclusion: HPV-DNA testing is expensive but very sensitive and exactly does what is required of a screening of diagnostic test. Despite its low specificity, this could be either addressed by running the test along with Pap smear test which has high specificity. The other alternatives to address the specificity are biomarkers.

TP/TTP in pregnancy

Rebecca Busch, Ashburton Hospital Laboratory

Thrombocytopenia in pregnancy occurs in up to 8% of patients, often due to haemodilution and increased platelet consumption/ aggregation in the third trimester. This condition is usually discovered incidentally during routine antenatal screening, however, more severe cases present with symptoms earlier in pregnancy. Untreated thrombocytopenia can place the infant at risk of intracranial or visceral haemorrhage and mother at risk of bleeding either during pregnancy or at birth.

Case study: Patient X presented on 14-01-09 feeling fatigued, with petechial rash, bleeding gums, microscopic haematuria and migraine headaches in the 15th week of pregnancy. Results of note in FBC - Platelets 8, Hb 83. Film showed schistocytes, polychromasis, red cell fragments(1%) .

The effect of haemolysis on Vitros 250 test results

Jackie Wright, Ashburton Hospital Laboratory

Purpose: ISO 15189 requires us to know what causes uncertainty in our test results, and to what extent. In updating our uncertainty data we discovered that information in our laboratory manuals on the effects of haemolysis on biochemistry test results was based on testing on a wet chemistry analyser.

Method: We undertook a small study to confirm the existing information was relevant to our laboratory's dry chemistry analyser.

Results: The results showed significant differences to the information on file. Haemolysis had no effect on dry chemistry magnesium and phosphate results. A subsequent review of haemolysed patient samples confirmed this; but also showed that other analyte level patterns did not always follow those noted in our study samples.

Conclusion: This exercise has reminded us to challenge "known" information by testing experimentally; and then retesting in real life situation. Haemolysis is multifactorial and not all causes and effects can be readily experimentally tested.

Life membership of the NZIMLS awarded to John Elliot

John Elliot trained at Wellington Hospital qualifying with "O" levels in haematology/blood bank and microbiology in 1967 and obtained an "A" level in microbiology the following year. He then spent a year at Hutt Hospital as a staff technologist before moving to India to work in the Kathleen Booth Hospital Laboratory. He returned to New Zealand in 1972 to take the charge position in microbiology at Hutt Hospital before moving in 1976 to take charge of microbiology at Wellington Hospital until 2000.

During the 70's he was at various times Chairman of the Wellington Branch of the Institute and was the Wellington region NZIMLS Council member between 1978 to1987. During his period on Council he was on its negotiation committee for industrial matters and played a key role on continuing education for the Institute's members. He represented Council on the State Health Employees Council and rose to its vice-president.

In 2000 he took up the position of Director of the Pacific Paramedical Training Centre in Wellington, a position he still occupies. During the last 10 years he has been instrumental in introducing and improving laboratory standards throughout the Pacific Island countries and has been extensively involved in teaching medical laboratory technologists throughout the Pacific Island countries both in-country and at courses run at the Pacific Paramedical Training Centre. Over the years he has acted as a consultant for the World Health Organisation in infectious diseases throughout the Pacific Islands and Asian countries.

Council is delighted to honour John Elliot with Life Membership of the NZIMLS for his long service to the profession.

Life Members of the NZIMLS

Colvin Campbell	Albert Nixon
Warren Dellow	Jan Parker
John Elliot	Desmond Philip
Shirley Gainsford	Dennis Reilly
Harry Hutchings	Trevor Rollinson
Michael Lynch	Gilbert Rose
Ron Mackenzie	Rob Siebers
Paul McLeod	Fran van Til
Kevin McLoughlin	Walter Wilson

Fellowship of the NZIMLS awarded to Andrew Stewart

Andrew Stewart has been awarded Fellowship of the NZIMLS following successful submission and examination of his treatise "HLA-B27 Polymorphism Associated with Disease in a New Zealand Population" which is published in this issue of the Journal.

Andrew is Acting Technical Specialist with the New Zealand Blood Service in Auckland. He works in the Tissue Typing Lab and his area of expertise is using DNA techniques for human leukocyte antigen (HLA) typing. He performs HLA typing for solid organ transplants, bone marrow transplants and diseases associated with HLA types. He has worked in Auckland for five years and previously worked in tissue typing with the Scottish National Blood Transfusion Service.

The Fellowship Committee congratulates Andrew on obtaining Fellowship, the Institute's highest professional qualification.

Rob Siebers FNZIMLS, Ann Thornton FNZIMLS, Jillian Broadbent FNZIMLS; Fellowship Committee, New Zealand Institute of Medical Laboratory Science

Fellows of the NZIMLS

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Journal questionnaire

Below are 10 questions based on articles in the November 2010 Journal issue. Read the articles fully and carefully, most questions require more than one answer.

Answers are to be submitted through the NZIMLS web site. Make sure you supply your correct email address and membership number. It is recommended that you write your answers in a word document and then cut and paste your answers on the web site.

The site has been developed for use with Microsoft's Internet Explorer web browser. If you are having problems submitting your questionnaire and you are using the Firefox web browser, try resubmitting from a computer or system using Microsoft's Internet Explorer.

You are reminded that to claim valid CPD points for successfully completing the Journal questionnaire you must submit an individual entry. It must not be part of a consultative or group process. In addition, members who have successfully completed the Journal questionnaire can only claim 5 CPD points. You can not then claim additional CPD points for reading the articles from which the questions were derived.

The site will remain open until Friday 18th February 2011. You must get a minimum of 8 questions right to obtain 5 CPD points.

November journal questions

1. Until recently, what have been the standard tools used for the diagnosis of malignant mesothelioma.
2. Malignant mesothelioma is a solid tumour of what and how does it usually present.
3. Name the cytologic features of mesothelial cells that are shared by reactive and malignant mesothelial cells.
4. Which genera that produce ESBL were resistant to all the antibacterial agents used in the study by Omoregie et al.
5. Isolates that produces ESBL are common causes of what in patients in acute care hospitals.
6. What is believed to be the major cause of mutation that has led to the emergence of ESBL.
7. HLA-B27 is strongly associated with which diseases and the spondylarthropathies affect which organs.
8. Which were the major HLA-B27 subtypes identified and what was the major HLA-B27 subtype in the Asian and European ethnic groups respectively.
9. What is macrocytosis generally attributed to.
10. What is the current view on the mechanism on what macrocytosis in COPD is due to.

Questions and answers for the August 2010 journal questionnaire

1. Name the three types of von Willebrand disease.
   Type 1 vWD is a partial quantitative deficiency in vWF, type 2 is a qualitative defect and type 3 is an extreme quantitative defect where vWF is absent.
2. What is the important role of the von Willebrand factor in primary heamostasis.
   Promoting platelet adhesion to sub endothelial structures such as collagen and platelet to platelet interaction following vessel injury.
3. Name the usual laboratory investigations in suspected cases of von Willebrand disease.
   Prothrombin time (PT), activated partial thromboplastin time (APTT) test, the platelet count, the bleeding time test and the platelet function assay (PFA).
4. What are the confirmatory tests for von Willebrand factor disorders.
   vWF:Ristocetin cofactor assay (vWF:RCo), the vWF antigen assay, the factor VIII coagulation activity assay and the collagen binding assay.
5. How may a patient suspected of von Willebrand disease present clinically.
   History of excessive bleeding or easy bruising and there may be some related family history.
6. By what principles do the vWF:RCo and the HaemosIL assays measure plasma von Willebrand factor.
   The vWF:RCo assay measures plasma vWF by binding vWF to the platelet gp 1b receptor on lyuphilised reagent platelets and agglutinates the platelets in the presence of ristocetin. Agglutinated platelets decrease reagent turbidity which is measured at OD^{405} nm with reduced light transmittance proportional to plasma vWF. In the HaemosIL assay plasma vWF is determined by turbidometric assay after it reacts with a monoclonal antibody anti-vWF (gp 1bα platelet binding site) bound to latex particles. Decreased turbidity is measured at OD^{405} nm.
7. What is vitamin D deficiency known to be associated among children and adults.
   Vitamin D deficiency is known to be associated with rickets among children and osteomalacia among adults.
8. What can highly elevated levels of vitamin D cause and what can it lead to.
   Highly elevated vitamin D levels can cause hypercalcaemia and, in the long term, can lead to renal stones.
9. What is the main form of vitamin D in circulation and which form of vitamin D becomes detectable when taking vitamin D supplements.
   25-hydroxyvitamin D is the main form of vitamin D in the circulation. 25-hydroxyvitamin D_2 becomes detectable when taking vitamin D supplements
10. What factors may cause interference in the Elecsys vitamin D (25-OH) assay.
    Factors that may cause interference with the Elecsys vitamin D (25-OH) assay are visible signs of haemolysis, gross lipaemia, gross icteric and highly elevated rheumatoid factors samples, and high doses of biotin.

Advertisers in this issue

The Pacific Way

Greetings to you all.
What has been happening at the PPTC lately?

Courses held at our centre in 2010

Biochemistry update

Again we are very indebted to the senior staff of the Dept of Biochemistry, Wellington Hospital for their contribution towards the biochemistry course which began on the 26th July and concluded on the 13th August 2010.

Biochemistry course students and PPTC staff

This course was an update in which participants learnt of recent advances in testing procedures relating to diseases of current interest in the Pacific. Topics included the following:

- Pre-analytical processes including: test ordering, blood collection systems, organisation of phlebotomy collection and laboratory reception
- IATA regulations for packaging of diagnostic samples and dangerous goods.
- Fundamental biochemistry equipment including, pipettes, pH meters, waterbaths, centrifuges, balances etc
- Renal function and renal dialysis.
- Blood gas analysers: their function and maintenance.
- Biochemical analysers including the Hitachi 902, Cobas C111, Minividas, DCA 2000 +, Piccolo.
- An overview of diabetes covering pathology, best laboratory practice in terms of diagnosis/ laboratory management and point of care testing.
- An overview of cardiac enzymes in health and disease with a focus on myocardial infarction and the best laboratory practice in terms of diagnosis and laboratory management of the disorder.
- Advances in protein analysis and specialist biochemical analysis.
- An overview of thyroid function and associated abnormalities.
- A molecular approach to biochemical pathology
- Quality control and external quality assessment in the biochemistry laboratory.
- The organisation and effective management of the biochemistry laboratory.
- The use of spreadsheets, and word processing in the biochemistry Laboratory.
- Discussion of quality systems, standard operating procedures, stock control / management, result processing / audit trails, reducing error and process improvement.
- An overview of Laboratory Information Systems.

The four participants who attended this course were: Kasian Otoko from Chuuk (Federated States of Micronesia), Mine Kojet from the Marshall Islands, Matias Da Silva from Timor Leste and Jeffery Vutilolo from Vanuatu.

The course was a tremendous success and the students carried home with them a huge amount of valuable information which they now hope to utilise effectively and improve the quality of their laboratory's diagnostic services.

Haematology and blood film examination

A second haematology course for 2010 was provided by PPTC for three students from Fiji namely Aralai Tuione from Suva, Arti Asheindar Kuar from Lautoka and Kaveesh Deo from Labasa.

This course is designed to give students confidence in the preparation, staining and examination of blood films, be able to differentiate the white cell count into both normal and abnormal populations and finally recognise and comment with confidence on abnormal blood film findings in an extensive range of common blood cell disorders.

The course provided students with guidelines for the objective microscopic evaluation of white cells, red cells and platelets in both health and disease. The students were introduced to the workings of the microscope in terms of correct operation, correct use of objectives, and essential maintenance. They learnt the principles of Romanowsky staining, the preparation of stains and buffers, causes of inconsistent staining quality, the correct staining techniques used in the identification of malarial parasites, specimen quality, the effects of anticoagulants, the correct technique in blood film making, morphological artefacts, buffy coat preparations and the correct storage of blood films. Students also developed an understanding of the relationship between clinical details, blood film findings and the automated full blood count. Morphological terminology with reference to origin and correct application was also discussed. The lineage of all blood cells was systematically followed from the common stem cell through all stages of development. Overall, a comprehensive account of both normal haematology and pathological haematology was given over the 4 week teaching programme.

The POLHN distance learning programme

This programme is provided and supported by the PPTC through WHO's POLHN and its lecturers are freely available on line to support all students through the programme if a theoretical or practical explanation is required. The programme is designed to qualify medical laboratory technicians in the medical laboratory sciences. The modules, however, are not only for new laboratory staff who wish to train as technicians, but also for qualified staff who wish to refresh or enhance their knowledge in the medical laboratory sciences.

A final opportunity to complete outstanding POLHN distance learning modules for the PPTC's Diploma in Medical Laboratory Technology has been offered to all students this year. Immunology

is the remaining module still to be offered this year, the starting date of which is the 1st November.

What has been planned for 2011?

2011 will see a change in course requirements in terms of the modules offered as well as the practical element of the POLHN programme.

- Projects will be replaced by the introduction of log books attached to each module for each discipline.
- Because the immunology module is smaller in content size when compared to the other four modules, it will now be incorporated into the microbiology and transfusion science modules allowing room for the creation of a new "laboratory technology" module.
- The theoretical content of each module will be updated and possibly expanded so as to accommodate current findings and newly developed technologies.

Students and staff of the 2nd haematology course 2010

The POLHN programme as it currently stands is very limited in the coverage of the basic sciences and therefore the PPTC is making an attempt to address this by the introduction of a newly devised laboratory technology module the content of which can be described as follows:

The laboratory technology module

The content will focus on:

- Biological structure and metabolic pathways of the living cell
- Cell division for growth and reproduction
- Elements and compounds, chemistry and life
- Organic molecules: the chemistry of carbon and hydrogen
- Amino acids and proteins
- Enzymes: catalytic proteins
- Lipids: fats and oils
- Carbohydrates
- Vitamins and minerals
- Human anatomy and physiology
- DNA, RNA and protein synthesis
- Laboratory calculations
- Basic laboratory equipment such as: balances, pH meters, pipettes, spectrophotometry, centrifuges, distillation, glassware, microscopes
- Chemical , electrical and biological safety in the laboratory

As from 2011, the Diploma course will require 2 years to complete and each module will be allocated 3-4 months in which to cover the content of the module as well as the completion of the log book.

Diploma modules to be offered in 2011

Feb	2011:	Laboratory technology
June	2011:	Haematology
Oct	2011:	Biochemistry

Diploma modules to be offered in 2012

Feb	2012:	Transfusion Sscience
June	2012:	Microbiology

Pacific travel

Fiji: Visit of Tirath Lakshman

Tirath is the is coordinator of the PPTC's Blood Bank EQA programme and in August he visited the laboratories at CWM Hospital, Suva and Lautoka Hospital to review the EQA programme with the staff and to also conduct some training as required. We thank Tirath for all the hard work that he does for the Pacific Island hospital labs and the PPTC in coordinating this programme.

Honolulu

John attended the PIHOA meeting in Honolulu from 30 August – 3 September. During the meeting there were sessions on education and training of health laboratory staff and also laboratory strengthening and capacity building. During these sessions John was able to discuss the various programmes that the PPTC has available in these areas and how they can be utilised to assist in meeting the needs of the laboratories in the north Pacific. While at this meeting a very useful meeting was held with Prof Ian Rouse, Dean of the School of Medicine, Health Sciences and Nursing [formerly FSM] and others regarding the recognition of the PPTC's DipMLT by the School. We should have more information regarding this in the coming months.

Fiji

WHO's strategy for strengthening health laboratories in the region

John and Phil attended a WHO meeting held in Fiji from 14th and 17th September. Attendees included representatives from most Pacific Island countries and in addition observers from NZAID, SPC, PIHOA, NRL and CDC. This meeting, at which the PPTC played a prominent role, acted as a forum to assist Pacific countries draw up national plans and policies for laboratories. A draft document proposing a regional standard for health laboratories was also introduced. The meeting was a very successful event and the guidelines to assist countries in the development and implementation of their national laboratory policy and strategic plan and for the regional standards are now in the process of being finalised and should be available for distribution to all countries early in 2011.

Courses for the remainder of 2010

1. ***Blood Transfusion Course:*** 18th October – 12th November. We are very grateful to Wellington NZBS staff who teach this four week course covering routine blood grouping, blood group antigens, crossmatch techniques, antibody detection, transfusion reactions, haemolytic disease of the newborn, blood donor selection, organisation of a blood bank and the appropriate use of blood components in transfusion medicine. Practical sessions are also provided, focusing on correct technique and fundamental basic procedures. One week of the course is set aside for an overview of current techniques in the detection of transfusion transmissible infections including HIV, syphilis and Hepatitis B and C.
2. ***POLHN Immunology module:*** 8th November

The Olympus Journal Imaging Competition 2011

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The NZIMLS Journal invites applications for the annual Olympus photo competition giving NZIMLS members the chance to win an Olympus digital camera and have their photo published in the journal.

The general theme is "Medical Laboratory Science", so whether it is related to haematology / histology, laboratory personnel, instruments, humour, or other, there's plenty of scope for keen photographers to showcase their talents.

Olympus, a leading manufacturer of professional opto-digital products, has generously donated a digital camera as the prize for the best photo.

Entries should be submitted as an email attachment to Rob Siebers, Editor of the NZIMLS Journal, at rob.siebers@otago.ac.nz A title for the photo, together with the entrant's name, place of work and email address, should accompany the attachment. Submissions can be in colour or black and white.

Entries close on **5pm on Friday 16th September 2011**, with the winning photo appearing in the November 2011 issue of the Journal.

Judging will be carried out by the Editor, Deputy Editor and an Olympus representative. Their decision will be final and no correspondence will be entered into. Entrants must be current financial members of the NZIMLS to be eligible.

For further information about the competition, go to: www.nzimls.org.nz

The winner of the 2010 Olympus Journal Imaging Competition is Lynne Pomare from Aotea Pathology, Wellington for her picture entitled "Uncertainty of measurement".

Reviewers for 2009/2010

The Editors would like to thank the individuals listed below for refereeing articles submitted to the Journal from September 2009 to August 2010. Some reviewers refereed more than once. All submitted articles undergo peer review in order that the Journal maintains its high standard. Additionally, thoughtful comments and suggestions made by referees help authors in ensuring that their article, if accepted, is put in front of the reader in the best possible light. The Editors and Editorial Board Members cannot be experts in all disciplines of medical laboratory science and thus rely on quality peer review by referees.

Not all articles submitted to the Journal are accepted for publication. In the last five years about 20% have been rejected as being scientifically unsound, not novel enough, not applicable to the broad subject of medical laboratory science or have previously been published in other journals. Duplicate publication is absolutely not allowed.

Reviewers 2009/2010

John Aitken, Christchurch
Robin Allen, Hamilton
Tony Barnett, Nelson
Chris Bowden, Christchurch
Russell Cooke, Wellington
Jaine Duncan, Christchurch
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Chris Kendrick, Palmerston North
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David Patterson, Christchurch
Holly Perry, Auckland
Rob Siebers, Wellington
Chris Sies, Christchurch
Lisa Stamp, Christchuch
Ann Thornton, Wellington
Lorna Wall, Auckland
Trevor Walmsley, Christchurch

We also thank the Journal's statistical adviser, Nevil Pierse, for his thoughtful review of statistics in submitted articles and Members of the Editorial Board for their advice and guidance.

Rob Siebers, FNZIMLS, Editor
Ann Thornton, FNZIMLS, Deputy-Editor
School of Medicine and Health Sciences, University of Otago, Wellington

New products & services

Medica is on the move.

After 12 years at Stoddard Rd Mt Roskill, Medica Pacifica is moving to the Auckland North Shore. A slight name change will also take place to coincide with the move. Medica Ltd will be relocating to 3 Te Kea Place Albany. The Pacifica name will remain as part of our Pacific Island business which is now based out of our Suva, Fiji office and warehouse. Please view our new website www.medica.co.nz for all the contact details.

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