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Submit all material electronically to the Editor at journaleditor1@nzimls.org.nz. Comprehensive instructions can be found in the NZ Journal of Med Lab Science 2000, vol. 54, issue 3, pages 108 to 110 or on the NZIMLS web site (www.nzimls.org.nz). When submitting provide a statement that the work is original, has not previously been published or is under consideration elsewhere, and that all named authors justify authorship by either contributing to the planning, execution, analysis and critical writing of the study, and all approve submission of the final version.

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New Zealand Journal of
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In this issue

The New Zealand poultry industry sparingly uses apramycin, an aminoglycoside antibiotic, to treat clinical cases of colibacillosis and infections caused by *E. coli* or *Salmonella* species. In this issue Jane Miller shows that, from supermarket brought chickens, *Escherichia* species from two chickens were found with apramycin, tobramycin and gentamicin resistances. The implication for humans is that they may be at risk from apramycin use in the poultry industry and suggests that there should be a ban of apramycin use in agriculture.

In an accompanying Editorial, John Aitken describes how Jane Miller, a student at St. Margaret's College in Christchurch, got to do her study on apramycin induced gentamicin resistant E. coli for her International Baccalaureate Diploma. Aitken states that Millar's simple but elegant findings *"raise serious questions about the lack of surveillance of retail food products in New Zealand."*

Rossi Holloway presents a case study of sideroblastic anaemia secondary to chronic alcoholism in this issue. Upon reducing alcohol intake and receiving folate and vitamin B6 therapy, some of the patient's haematological abnormalities had returned to normal. Holloway then gives a brief overview on the haematological aspects of chronic ethanol consumption and associated sideroblastic anaemia.

In this issue Gloria Evans gives a comprehensive review of molecular methods used in medical microbiology testing. Of particular interest to New Zealand medical laboratory scientists is an up to date list of direct molecular testing on clinical specimens currently available in New Zealand medical laboratories.

Dennis Reilly, from MedLab Auckland, delivered the prestigious TH Pullar Memorial Address at the South Pacific Congress in August. His address is in this issue and Reilly, among other issues, comments on the recent events surrounding the Auckland medical laboratory scene. He states that there are still challenging times ahead for laboratory services in New Zealand.

Another journal-based questionnaire is in this issue. As with the previous issue's questionnaire, members will have to get at least 8 out of 10 questions right to earn 5 CPD points. The journal-based questionnaire is a very popular avenue for members to obtain CPD points, with over 500 members submitting for each of the past issues' questionnaires. However, there were some members who initially did not get at least 8 answers right from the August 2007 issue. Looking at those, as well as many where members just managed to get 8 out of 10 right, it appears that some members do not read the articles or the questions carefully. Remember, most questions require more than one answer.

To the Editor:

In my recent article "The diagnostic footprints – a case study" (*N Z J Med Lab Sci* 2007; 61 (2): 33-5) I forgot to put in the following acknowledgement:

The actual protocol for checking reaction monitors on HDL cholesterol results of <0.50mmol/L, is an NZDG wide protocol, originating from work initiated at Southern Community Laboratories, Christchurch by Maxine Reed.

Barbara Hoy

Hamilton

Med-Bio Journal Award

med·bio

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

Winner of the Med-Bio Journal Award from the August 2007 issue was Rajani Gutha from Core Laboratory, Wellington Hospital for her article "Serial serum prostate specific antigen measurements over time in a patient presenting with a metastatic adenocarcinoma of unknown origin". *N Z J Med Lab Sci* 2007; *61 (2): 31-2.*

Editorial

Heigh-ho! Come to the fair!

It's that time of year again, the birds are singing, the daffodils are blooming, and across the country the school science fairs displays are opening their doors to the public. Concurrently supermarkets enjoy increased sales in cleaning products, Ribena™, dishcloths and antibacterial toilet cleaners while local fast-food retailers quake in fear.

The first sign of spring for the medical laboratory is the phone calls from parents asking if we can help in management of their child's project. I generally commence this process by meeting with the child, to assess the attitude of the researcher, the resources required and the scope of the project. Projects range from the mundane (the much favoured testing of the advertisers' claims to eradicate "99.9% of all household germs") to the frightening. One ambitious 14 year old wanted to transfer plasmid-mediated resistance to important human pathogens and in response to my obvious reluctance, pointed out that there was nothing against genetic modification in the science fair regulations! We discussed ethics at some length, and the subsequent project, whilst a shadow if its former self, still won a prize, and the world is safer for democracy.

And then there are the children, and they fascinate me. Encouraged by parents, teachers, the Discovery Channel, an early encounter with a chemistry set or a toy microscope, they follow their dream. If they are very lucky, they experience at a young age what Richard Feynman (1) calls "the kick in the discovery", and they are addicted for life.

Like the alien-implanted characters portrayed in "Close Encounters of the Third Kind", they embark on the search for the Mother Ship. Eventually they arrive at our doors, and hopefully we welcome them, for their passion has driven them a long way. Crown Research Industries (CRIs) do not feature prominently as science fair mentors, perhaps they are restricted by health and safety concerns in their interactions with schoolchildren, or do not welcome the opportunity to create controversy. Some of the projects challenge advertising claims or government wisdom. Projects require supervision, and most medical laboratories are understaffed and overworked, but we do two things well : we teach, and we produce data.

Nearly all the data available to the Government for planning of preventative health strategies (sexually transmitted diseases, diabetes, heart disease, antibiotic resistance) is produced by the medical laboratories. Subsequent press releases by high profile researchers and the CRIs may imply otherwise, but reliance on the medical laboratories is crucial to the tediously constructed monitoring reports and the conclusions reached by research bodies.

Medical laboratories are also the prime source of technical workers for food and veterinary laboratories, CRIs, State Owned Enterprises (SOEs), environmental laboratories, and all levels of scientific research. Additionally, medical laboratory scientists train clinical microbiologists and Bachelor of Medical Laboratory Science (BMLS) students. When we are gone, they will miss us.

Jane Millar is an example of what we do best, and her work is material evidence of her ability to analyse data, develop methodologies, and to think for herself. I first met Jane Millar when, as a 13 year old student, she shared our laboratory resources to complete a science fair project. Her contribution to this issue of the Journal was carried out when she was 17 years old, as part of a Baccalaureate qualification, and is a good example of the type of research carried out by High School students throughout New Zealand.

Millar describes the steps taken to isolate antibiotic resistant *E.coli* from retail chicken products and discusses the relevance of her findings in relation to the use of antibiotics by the poultry industry (2). The technical simplicity of this study and the process by which she analysed her observations raise serious questions about the lack of surveillance of retail food products in New Zealand. Eight years ago the Animal Remedies Board and it's successor, the New Zealand Food Safety Authority (NZFSA), were charged with the responsibility of monitoring antibiotic resistance in retail meats (3), but this is yet to happen. Perhaps the NZFSA should be going to High Schools and science fairs to contract for the necessary research!

Richard Feynman observed that "science is truth" (1) and Millar's successful isolation of aminoglycoside resistant *E.coli* from domestic retail chicken product is indisputable. Her concern about contamination of retail meat with antibiotic resistant *E. coli* is well-founded, and the conspicuous lack of independent research in New Zealand has been partially addressed by her intelligent speculation on the circumstances surrounding her primary observations.

Millar's observation that poultry products may be a medically important source of antibiotic resistance factors in *Enterobacteriaciae* is not new, the most recent example is reported in a study by Johnston et al (4) and describes the genesis and likely transmission route of antibiotic resistant E.coli from poultry to humans in the United States of America. Certainly, medical laboratory scientists in New Zealand are familiar with the faecal-oral route as a significant mode of transmission for multiply-antibiotic resistant *E.coli* within healthcare facilities in New Zealand.

Given also the high rates of food-borne gastrointestinal disease in New Zealand it is highly likely that gentamicin resistant *E.coli* from poultry are capable of colonising the human gut. It is but a short step in imagination to envision widespread dissemination and plasmid-mediated transmission of these resistance factors to other gram-negative enteric bacilli.

For all the hype about the "knowledge wave", innovation in science and the pursuit of excellence in research, most scientists measure government support in terms of financial support and therefore have a justifiably pessimistic attitude to the future of science in New Zealand. Behavioural and social science is favoured in funding rounds. Environmental and diagnostic microbiology research may result in bad news for the Government. As J Edward Deming observed, and scientists echo, "bearers of bad tidings seldom fare well." The lips of the Emperor say "yes" to investigative environmental microbiology but the eyes scream "no!" The tide, it seems to some, is going out on science in New Zealand and the future career prospects for budding young scientists are bleak. Training and supportive infrastructure have been eroded away by successive waves of reform calculated to extract maximum short-term value for the science dollar.

In the midst of such gloomy and apocalyptic speculation, there are some rays of hope. There are teachers who champion science in the classroom, and convey their evangelical zeal like electricity to receptive students. In the face of the tedium of the education system they stand out. Their untiring efforts are celebrated at the science fairs and across the family dinner tables of their pupils.

The children who shine in science fairs and baccalaureate projects seem to have a passion for science and often exhibit a perseverance and single-mindedness towards their work that is rejuvenating for

those of us who are soon to leave the bench. In their future careers (should they be fortunate enough to find a job in science which realises their dreams) they hopefully will not take "no" for an answer, and will not compromise in the face of political pressures to back away from controversial issues. Hopefully.

Maybe, as the British rock group "The Who" put it 40 years ago : "The Kids are Alright"

References:

1. The Pleasure of Finding Things Out. Richard P Feynman. Penguin Press, 2000. ISBN 0-14-029034-6.

2. Millar JR. The relationship between use of apramycin in the poultry industry and the detection of gentamycin resistant E. coli in processed chickens. *N Z J Med Lab Sci 2007; 61: xx-xx.*

3. Antibiotic Resistance Steering Group. Report to the Animal Remedies Board, 22 September 1999.

4. Johnson JR, Sannes MR, Croy C, Johnston B, Clabots C, Kuskowski MA, et al. Antimicrobial drug-resistant Escherichia coli from humans and poultry products, Minnesota and Wisconsin, 2002-2004. *Emerg Infect Dis* 2007; 13; 838-46.

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NZIMLS Journal Prize

Council of the NZIMLS has approved an annual Journal prize for the best case study accepted and published in the Journal during the calendar year. The prize is worth $200.

Case studies bring together laboratory results with the patient's medical condition and are very educational. Many such studies are presented at the Annual Scientific Meeting, SIG meetings, and the North and South Island Seminars, yet are rarely submitted to the Journal for wider dissemination to the profession. Consider submitting your case study presentation to the Journal. If accepted, you are in consideration for the NZIMLS Journal Prize and will also earn you additional CPD points. Please contact the Editor or any Editorial Board Member for advice and help. Contact details are on the NZIMLS web site (www.nzimls.org.nz) as are instructions to authors.

No formal application is necessary but you must be a financial member of the NZIMLS during the calendar year to be eligible. All case studies accepted and published during the calendar year (April, August and November issues) will be considered. The Editor, Deputy Editor and President of the NZIMLS will judge the eligible articles in December each calendar year. Their decision will be final and no correspondence will be entered into.

The relationship between use of apramycin in the poultry industry and the detection of gentamicin resistant *E. coli* in processed chickens

Jane Rosemary Millar, International Baccalaureate Diploma
St. Margaret's College, Christchurch

Abstract

Apramycin is an aminoglycoside antibiotic used sparingly by the New Zealand poultry industry. Apramycin resistance is generally caused by an aminoglycoside modifying enzyme (AME), with the most common example being apramycin-acetyltransferase [AAC(3)-IV]. This enzyme can also inhibit the action of two common and medically important aminoglycosides, tobramycin and gentamicin. Development of apramycin resistance requires previous exposure to the compound, so consequently resistance to all three aminoglycosides can be triggered by exposure to apramycin. Research has found that antibiotic resistant bacteria can be introduced to the human gastrointestinal tract through food, where they can spread their resistance factors to other bacteria via horizontal gene transmission. Acquired antibiotic resistance in pathogenic bacteria prolongs infections and decreases treatment options.

Gram-negative bacterial strains were cultured from six different raw chickens from a variety of commercial sources, including organic farms. The susceptibility of these strains to a range of medically important aminoglycosides and apramycin was established using the Kirby-Bauer disc diffusion method. After incubation, the resulting zone sizes were measured to establish the susceptibility range.

Escherichia species from two chickens were found with apramycin, tobramycin and gentamicin resistances. This observation suggests that the resistance resulted from prior exposure to apramycin, causing resistance due to the acquiring of the [AAC(3)-IV] enzyme. This pathway is significant, as it suggests humans are at risk from apramycin use in the poultry industry.

Key words: apramycin, gentamicin resistant *E. coli*, poultry

N Z J Med Lab Sci 2007; 61 (3): 65-8.

Introduction

Chicken represents 35 percent of all meat consumed in New Zealand (1). In 2006, the average New Zealander consumed 36.5kg of chicken (2). To supply this heavy demand, New Zealand poultry farmers have adopted methods of intense chicken husbandry, minimizing the use of land space. However, this can lead to the chickens being raised in cramped conditions enabling rapid transmission of pathogenic bacteria within a flock. One way to control this spread is the sub-therapeutic use of antibiotics. Antibiotics are administered to flocks of chicken through feed or water, acting not only as general disease-prevention insurance, but also as a growth promoter. The mechanism for the latter is not well-understood, but may result from the reduction of normal intestinal flora which compete with the host for nutrients. Harmful gut bacteria are also reduced, which may indirectly promote growth. Prolonged use of low levels of antibiotics may select and promote the growth of antibiotic resistant bacteria in the host..

Apramycin, an aminoglycoside is important in animal husbandry around the world and is used by the Poultry Industry Association of New Zealand (PIANZ) members, who collectively account for 99% of New Zealand's broiler chicken production. Apramycin is predominantly used in poultry breeder flocks for short periods, to treat clinical cases of colibacillosis and infections caused by *E. coli* or *Salmonella* species. During the last audited year (2004-2005) only 7.35 kilograms of apramycin was administered to chickens belonging to PIANZ; a mere 0.014% of all antibiotics used in New Zealand animals (3).

Gentamicin has been used in horticulture and agriculture in New Zealand (4), PIANZ states that their members have never used gentamicin (3). Tobramycin is an aminoglcoside with an extended spectrum capable of treating infections with gentamicin resistant bacteria. It is not used in agriculture, but is reserved for life-threatening infections in humans.

Although there are different mechanisms of aminoglycoside resistance, plasmid encoded enzymatic modification is the most common (5). Many aminoglycoside modifying enzymes (AME) exist and by testing the susceptibility of isolates against a range of aminoglycoside antibiotics, a pattern of resistance emerges that is unique to a specific enzyme (6). A commonly known apramycin inhibiting enzyme is apramycin-acetyltransferase [AAC(3) -IV] which also inactivates gentamicin and tobramycin (7). The resistance for apramycin due to this enzyme is unique in that it only occurs after exposure to apramycin. Interestingly, exposure to apramycin can cause the resistance to apramycin, gentamicin and tobramycin, but gentamicin exposure cannot cause apramycin resistance to form (J Aitken, personal communication).

Methods

6 fresh chickens were purchased from Christchurch Merivale Fresh Choice supermarket on the 16th February 2006 where they had been displayed in an open 4°C refrigeration unit. Each chicken was assigned a number to ensure anonymity of the producer. These chickens were:

1. Corn-fed free-range fresh chicken.
2. Fresh chicken, barn raised, no added hormones.
3. Fresh chicken, no added hormones, barn raised, size small.
4. Chicken, free range organic "bio-gro" certified, no growth promotants, no antibiotics
5. Fresh chicken, no added hormones, barn-raised .
6. Fresh chicken, no hormones added, barn raised, size large.

Colonies of Gram-negative bacteria were isolated from the different brands of raw chickens using selective media and the aminoglycoside susceptibility profiles were determined using the Kirby-Bauer method as follows:

Using a sterile syringe, the plastic wrapping was punctured and approximately 100mL of the fluid was aspirated from each chicken and transferred into a corresponding numbered test tube. 100µL of the fluid from each chicken was transferred into five sterile test tubes containing MUG broth (a qualitative fluorescent detection media used to detect *E.Coli* in water samples). The tubes were then incubated for 24 hours at 37°C with lids on.

After removal from the incubator, in a dark area a UV light was shone over each test tube. Any fluorescence was recorded and a blood MacConkey agar plate was inoculated and streaked for each test tube (i.e. 5 plates for each chicken). The plates were incubated for 24 hours at 37°C.

One sample colony of growth on each plate and was emulsified in a nutrient broth and this was used to prepare KB antibiotic sensitivity plates stamped with apramycin, gentamicin, tobramycin, kanamycin, streptomycin, neomycin, and amikacin discs, then incubated for 24 hours at 37°C. The antibiotic susceptibility plates were examined after incubation and susceptibility profiles were measured and recorded. Isolates exhibiting apramycin resistance were observed and a representative isolate was taken for further study. The antibiotic disc zone sizes on the plates were measured and CLSI based methodology was used to classify the bacteria as being resistant, intermediate or sensitive to the antibiotic. API20 bacterial identification kits were used to identify the genus and species of any growths showing significant aminoglycoside resistances.

Additionally, 5ml of saline was used to wash the chicken carcass and then centrifuged at 3000rpm for 15 minutes. The centrifigate was inoculated onto *Campylobacter* isolation media (Fort Richard) and incubated at 42°C for 48 hours in CO2. After incubation the plates were examined for growth of *Campylobacter* species.

Results

Campylobacter species were isolated from all six chickens. All samples fluoresced under the UV light, indicating the presence of *1* but not the quantity. The different chickens' isolate resistance patterns were as shown in Figures 1 to 7.

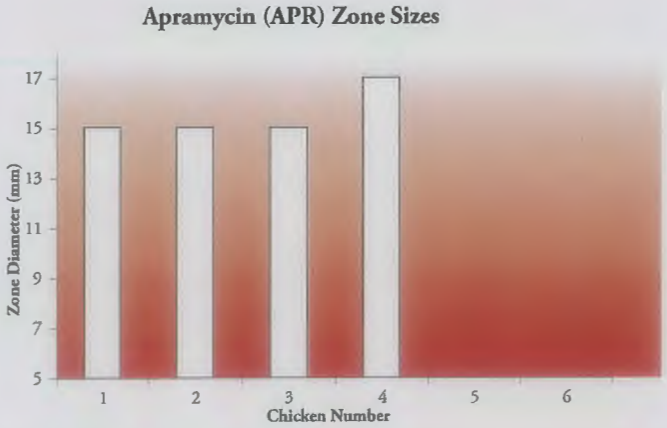

Figure 1. Apramycin zone sizes.

Figure 1 shows that the bacteria from chickens 5 and 6 were resistant to apramycin while the rest were sensitive. No intermediate resistances were found, indicating that apramycin resistance factors cause complete resistance.

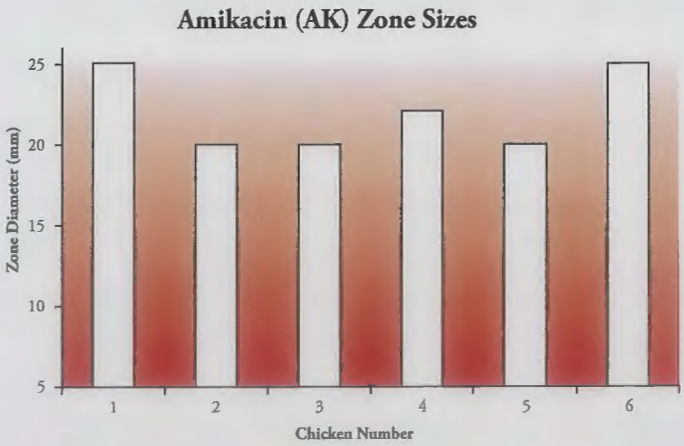

Figure 2. Amikacin zone sizes.

Figure 2 shows that all the bacteria were sensitive to amikacin. This is to be expected, as amikacin is not used in agriculture. The lack of resistance also indicates that the AME causing amikacin resistances is not formed in response to apramycin exposure.

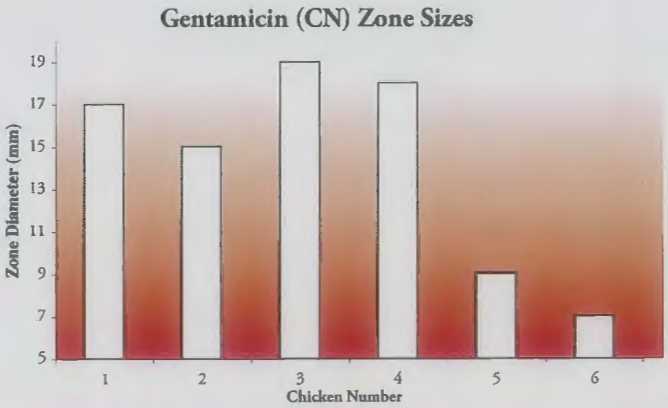

Figure 3. Gentamycin zone sizes.

Figure 3 shows that the susceptibility for gentamicin varied considerably between the bacteria from the different chickens. The bacteria from chicken 5 and 6 were considered to be resistant because of the limited zone size. The rest were classified as sensitive. Since gentamicin is not used in agriculture, resistance cannot have been caused by exposure. Gentamicin resistance only occurred where apramycin resistance occurred (Comparing Figures 1 and 3). Apramycin and gentamicin resistances are known to be caused by the same AME, [AAC(3)-IV], therefore these gentamicin resistances probably exist due to apramycin exposure.

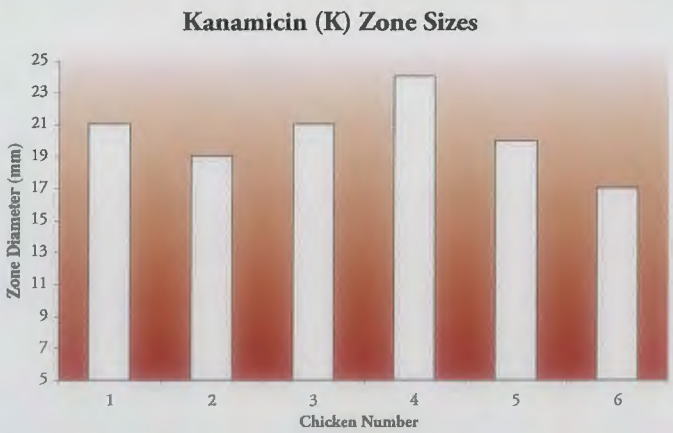

Figure 4. Kanamicin zone sizes.

Figure 4 shows that the bacteria cultured from all of the chickens were sensitive to kanamicin. This is to be expected, as kanamicin is allegedly not used in agriculture. The lack of resistance also indicates that the AME causing kanamicin resistance is not formed in response to apramycin exposure.

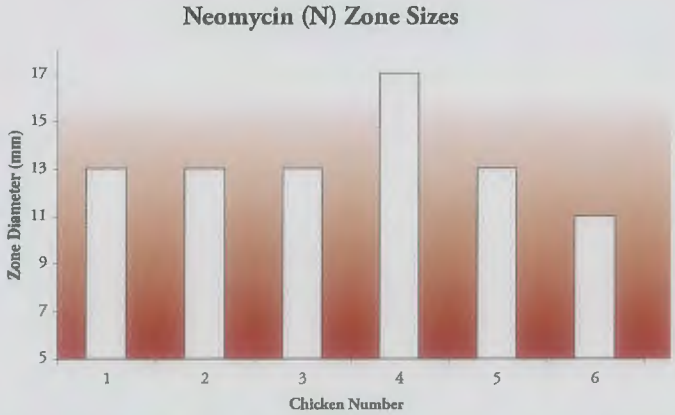

Figure 5. Neomycin zone sizes

Figure 5 shows that the majority of the bacteria were intermediate resistant to neomycin. Since neomycin is registered for use by PIANZ[1], this intermediate resistance probably resulted from neomycin exposure.

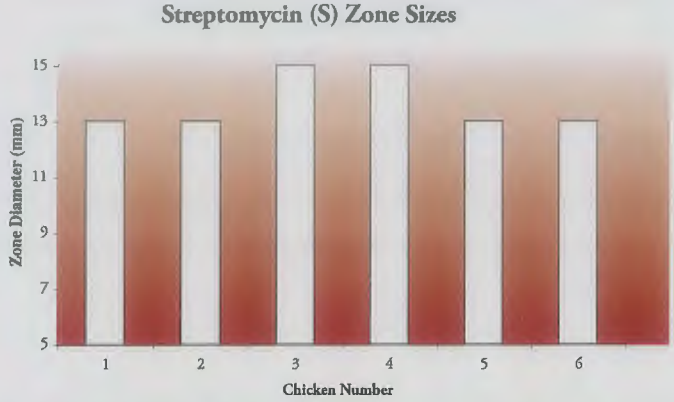

Figure 6. Streptomycin zone sizes.

Figure 6 shows that there was little variation in resistance to streptomycin and that the majority contained bacteria intermediately resistant. Streptomycin is not used by PIANZ and it is not believed to be a substrate of the AME caused by exposure to apramycin. Streptomycin, however, is used in large amounts in the pip fruit industry and this activity could result in a significant reduction in streptomycin sensitivity in the environment that the chickens may have acquired.

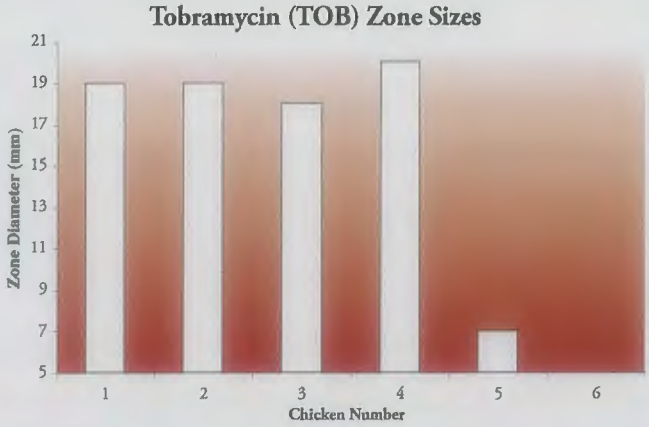

Figure 7. Tobramycin zone sizes.

Figure 7 shows the large variation in resistance to tobramycin. The bacteria from chicken 6 was completely resistant to tobramycin, the bacteria from chicken 5 had a very restricted zone size, classifying it also as resistant, while the rest of the bacteria was sensitive. Since tobramycin use is restricted to human medical use only, resistance cannot have been caused by exposure. Tobramycin resistance only occurred where apramycin and gentamicin resistance occurred (comparing fFgures 1, 3 and 7). Therefore these tobramycin resistances probably were caused by apramycin exposure causing a resistance due to the [ACC(3)-IV] enzyme, resulting in apramycin, gentamicin and tobramycin resistances.

Campylobacter species was grown from all six samples, including the organically raised chickens. Results from the Api20 test kits identified the bacterial isolate from chicken 5 as *Escherichia coli* and chicken 6 as *Esherichia fergusonii.*

Discussion

In both apramycin resistant isolates, resistance was found to gentamicin and tobramycin, medically important antibiotics. Since apramycin, tobramycin and gentamicin resistance can all be caused by the production of the [AAC(3)-IV] enzyme in the bacterium due to apramycin exposure, these results suggest that the use of apramycin by PIANZ has triggered medically important aminoglycoside resistant bacteria in chickens.

Campylobacter is one of the most of common causes for human gastroenteritis, and its occurrence in all 6 sampled chickens is unsurprising, given the established link between Campylobacter infection and raw chickens.

In 2003 Cook et al (8) examined vancomycin-resistant *Enterococcus facecalis* isolates in humans, which appeared to be indistinguishable or genetically closely related to the dominant poultry vancomycin-resistant *E. faecalis* clone. It was suggested that there was either a transfer of antibiotic resistant bacteria from poultry to humans by the food chain, or gene transfer of resistance in the human gastrointestinal tract. Similarly it is possible for the aminoglycoside resistant bacteria found in this investigation to be introduced into the human gastrointestinal tract where they can survive for up to six weeks (9), enabling gene transfer to take place. This can lead to the transference of resistance to other pathogens, thus preventing the possibility of therapeutic treatment for that pathogen with the aminoglycoside antibiotics. If infections caused by resistant bacteria fail to respond to treatment it can result in prolonged illness and a greater risk of death. Failed treatment can also

mean longer periods of infectivity, increasing the number of infected people in the community. And finally when infections become resistant to first-line antibiotics, second or third-line drugs are used, which are nearly always much more expensive and sometimes more toxic as well.

The acquisition of resistant plasmids may have been a consequence of selective pressure exerted by the use of apramycin in poultry. The two isolates which are assumed to contain the [AAC(3)-IV] AME belong to the *Escherichia* genus, which are ubiquitous inhabitants of the intestine of warm-blooded animals, including avian species and are usually harmless. The resistant strains were identified as *E. coli,* and *E. fergusonii*. These bacteria are potential pathogens as new strains arise all the time from natural mutations including particularly virulent strains such as *E. coli* 0157:H7. Effective use of antibiotic therapy will result usually in the eradication of the infecting organism, therefore the presence of resistant bacteria suggests that bacteria survived apramycin exposure through a natural selection process caused by sub-therapeutic use.

Finally, apramycin is used mostly for infection control of breeder flocks of chickens, which are not processed for consumption. Finding the resistant bacteria in broiler chicken implies spread of bacteria between flocks, suggesting that the consequences of antibiotic use are more widespread than anticipated.

Apramycin resistant bacteria may have been developed by intensive use of apramycin previously, so may not have been caused by the current therapeutic use. This is a possibility because antibiotic resistant bacteria can persist long after the removal of the selection pressure (10). Aminoglycoside resistances that have arisen may therefore be difficult, if not impossible to reverse. A comprehensive field evaluation of apramycin use by PIANZ would be needed to further investigate this likelihood. If these resistances were caused by the historic use of apramycin, it would be a strong indicator that the use of apramycin causes other resistances to develop.

PIANZ justify the use of antibiotics by saying the chickens must be *"protected from and, the rapidly diagnosed of any significant injury of disease."* (11). According to PIANZ, when used for health maintenance, antibiotics allow better nutrient utilisation and reduce the amount of feed needed and allow the chickens to grow to full potential. Antibiotics also lower the incidence of sickness and death in the chicken flocks, consequently reducing the pain and suffering of the chickens. PIANZ have stated *"To date, there is only indirect scientific evidence linking the use of antibiotics in food animals with the potential to compromise the efficacy of related antibiotics in humans."* and present results from overseas studies (conducted by the Heidelberg Appeal Netherland Foundation 1999), which found that there was no evidence that antibiotics used in animal production compromise the efficacy of related antibiotics in human medicine. In my opinion, the small sample of this investigation contradicts their claim, raising the possibility that industry may select scientific information displaying results benefiting their business.

Intermediate resistances to neomycin and streptomycin were also found but suggested a different resistance mechanism than the AME caused by apramycin exposure. The neomycin resistance may have been caused by its use in the poultry industry and the streptomycin resistance may have been caused by use in horticulture. Not enough is known about the resistance mechanisms of neomycin and streptomycin to make any definitive links to use in agriculture.

Streptomycin is not used by PIANZ, but is extensively used in other areas of agriculture and horticulture. The discovery of streptomycin resistances in the chicken has indicated the extent of the possible widespread distribution of antibiotic resistant bacteria. The transmission of antibiotic resistant bacteria is not limited to food-borne transmission; other methods include surface water via eating crops and meat pet food via pets. These methods have not been investigated in-depth, so are poorly understood, but still pose a significant risk to human health.

The Expert Panel on Antibiotic Resistance convened by the New Zealand Food Safety Association (NZFSA) in 2005 did not express concern about aminoglycoside use in poultry because it was used minimally and in highly supervised conditions (3). However, an external review of the Expert Panel's review said, *"There is no discussion of the strong cross-resistance relationship between apramycin and gentamicin."* (12). It is possible that the NZFSA were unaware of the environmental impact of apramycin, or that the pressure to supply the growing population's food demand is too overwhelming to consider other options. Either way, there has not been enough monitoring or investigation of apramycin usage for satisfactory answers to the questions raised by this research, which suggests that restricted use of apramycin, even for therapeutic purposes cannot guarantee that resistance to aminoglycosides important in the human medicine will not be selected.

In order to preserve and protect the effectiveness of medically important antibiotics, the spread of antibiotic resistant bacteria needs to be reduced. This should start with a ban of apramycin use in agriculture.

References

1 The New Zealand Herald. Playing chicken with your health. 14 July 2007. http://www.nzherald.co.nz/section/story.cfm?c_id=204&objectid=10451424&pnum=0.

2 Poultry Industry Association of New Zealand (Inc). http://www.pianz.org.nz.

3 Gerber N. PIANZ (Inc). 30 June 2006.

4 New Zealand Food Safety Association. Antibiotic resistance and in-feed use of antibiotics in New Zealand. http://www.nzfsa.org.nz.

5 University of Pennsylvania Health System (online) updated 2006 http://www.uphs.upenn.edu/bugdrug/antibiotic_manual/aminoglycosideresistance.html .

6 Mingeot-Leclercq MP, Glupczynski Y, Tulkens PM. 1999 Aminoglycosides: activity and resistance. *Antimicrob Agents Chemother* 1999; 43: 727-37.

7 Gómez-Lus R. Evolution of bacterial resistance to antibiotics during the last three decades. *Int Microbiol* 1988; 1: 279-84.

8 Cook GM, Manson JM, Smith JMB. Time to ban al non-therapeutic use of antimicrobials in New Zealand animals *N Z Biosciences* 2003.

9 Sorensen TL, Blom M, Monnet DL, Frimodt-Moller N, Poulsen RL, Espersen F. Transient intestinal carriage after ingestion of antibiotic-resistant *Enterococcus faecium* from chicken and pork. *N Engl J Med* 2001; 345:1 161-6.

10 Chaslus-Dancla E, Gerbaud G, Lagorce M,. Lafont JP, Courvalin P. 1987 Persistance of an antibiotic resistance plasmid in intestinal *Escherichia coli* of chickens in the absence of selective pressure. *Antimicrob Agents Chemother* 1987; 31: 784-8.

11 Animal Welfare Act 1999. http://www.pianz.org.nz.

12 Turnidge J. Review of report by the expert panel on antibiotic resistance "a review of the impact of the use of antimicrobials in animals and plants on the development of antimicrobials resistance in human bacterial pathogens", FRACP, FRACPA 2005.

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Sideroblastic anaemia secondary to chronic alcoholism: a case study and review

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Abstract

Recent literature on secondary acquired sideroblastic anaemia reports on the high prevalence of this type of anaemia in alcoholics. Studies have also shown that the sideroblastic abnormality is only seen in those alcoholics with an associated folate deficiency. Furthermore, folate deficiency in alcoholics is not only from the poor nutrition associated with a 'liquid diet', but also from the effect of ethanol on folate absorption, catabolism and excretion. This case study is used to review recent literature on the haematological aspects of chronic ethanol consumption and associated sideroblastic anaemia.

Key words: sideroblastic anaemia, alcohol, folate
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Case history

Patient B, a 54-year-old female, presented to the emergency department in June 2006 with a moist cough and coinciding chest pain, and possible jaundice. Emergency staff noted a 'stale alcohol smell' on admission. Examination showed a weight of 49 kg and an enlarged liver. The patient stated that she had lost approximately 25kg over the last two years. She was admitted to the ward and intravenous antibiotics for the treatment of pneumonia were begun. On admission, the patient had a macrocytic anaemia (MCV 108 fL and Hb 106 g/L), with target cells and toxic changes seen in the blood film. She had abnormal liver functions with a markedly raised GGT of 1114 U/L.

The patient presented again December 2006 with symptomatic anaemia, and investigation showed that she consumed 10-15 nips of whiskey a day. Clinical investigations ruled out blood loss as a cause of her anaemia. Her folate level was 3.6 nmol/L, and she had an absolute reticulocyte count of $9x10^9$/L. Siderocytes had been noted on prior blood film examinations but it was not until this time that alcoholism was suggested as a cause, and that sideroblastic anaemia could be present in this patient. In review of these results, a bone marrow aspirate was recommended. This showed mixed marrow cellularity, with iron stores 4+ (using Prussian blue staining), and showing florid sideroblastic change. At the time of this bone marrow aspiration, Patient B had ferritin of 2049 g/L and her iron saturation was 73%. Both of these extremely high results were due to her sideroblastic abnormality. A diagnosis of alcoholic liver disease with secondary acquired sideroblastic anaemia was therefore established for this patient.

Patient B finally presented in March 2007 with a H of 70 g/L. She had dramatically reduced her alcohol intake and had been on folate and vitamin B6 therapy. Her MCV had returned to within normal range (96 fL), and no siderocytes were seen in her blood film.

Sideroblastic anaemia

The sideroblastic anaemias are a heterogenous group of disorders with the unique characteristic of amorphous iron deposits in erythroblast mitochondria (1). These iron deposits are the result of ineffective insertion of iron into the developing haem molecule. To understand the causes of sideroblastic anaemia, the pathway of haem synthesis is described in Figure 1.

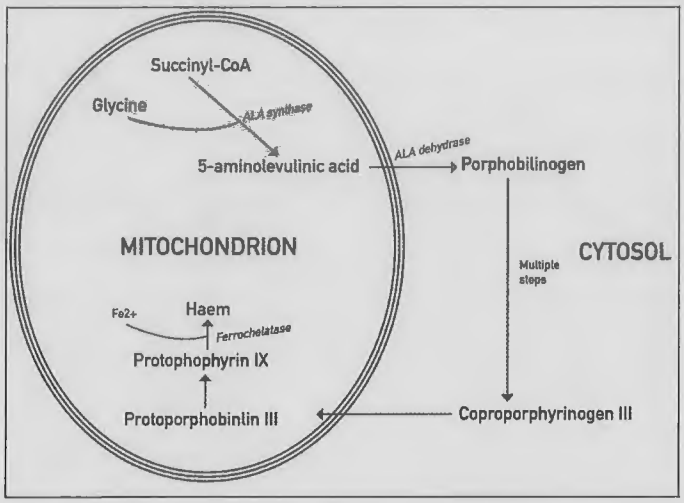

Figure 1. Simplified diagram of haem synthesis, occurring both within and outside the mitochondrion. Note that the cofactor for ALA-synthase is pyridoxal phosphate. ALA: 5-aminolevulinate. Modified from Bridges, and Wiley & Moore (2,3).

There are three forms of sideroblastic anaemia: hereditary, primary acquired, and secondary acquired. The hereditary form is caused by a mutation on the X-chromosome, resulting in a defect in ALA-synthase (Figure 1). Primary acquired sideroblastic anaemia, which involves abnormal haematopoiesis, is idiopathic, as it is not clear what the cause of defective haem synthesis is in the abnormal erythroid clone (3) This type of sideroblastic anaemia is the one most commonly seen in the haematology laboratory, as it is part of the myelodysplastic syndromes – a series of clonal haematopoietic stem cell diseases. The myelodysplastic syndromes are characterised by refractory anaemia and ringed sideroblasts, which can transform into a state with excess blasts and then on to acute leukaemia.

The causes of secondary acquired sideroblastic anaemia, and the mechanisms behind them, have been extensively researched. One of the most common causes of secondary acquired sideroblastic anaemia is chronic ethanol consumption, with a ringed sideroblast abnormality occurring in 25-30% of anaemic alcoholic patients (1,4,5). Consumption of over 80g ethanol a day will lead directly to sideroblastic anaemias (1). A single 45 mL nip of whiskey contains 12 g ethanol, so to consume >80 g/day, the patient must be drinking over 6 nips a day (6).

Alcohol has two effects on haematopoiesis. Firstly, it is directly toxic to developing cells, causing an increased or high-normal MCV and vacuolation of erythroid precursors. Secondly, alcohol consumption causes lowering of the plasma concentration of pyridoxal phosphate. As shown in the pathway of haem synthesis (Figure 1), pyridoxal phosphate is a cofactor for ALAS. The product of alcohol breakdown in the liver, acetaldehyde, accelerates hepatic degradation of pyridoxal phosphate, meaning there is less cofactor for ALAS and thus the pathway of haem synthesis is disrupted (3). As a result, iron is not inserted into the

developing haem molecule, but builds up in the mitochondria. Because erythrocyte production is depressed, more iron is absorbed from the gastrointestinal tract in an attempt to increase this. Thus, the patient moves into a state of iron overload. If alcohol consumption is ceased, the sideroblastic abnormalities in these patients will resolve within 14 days (1,3,4).

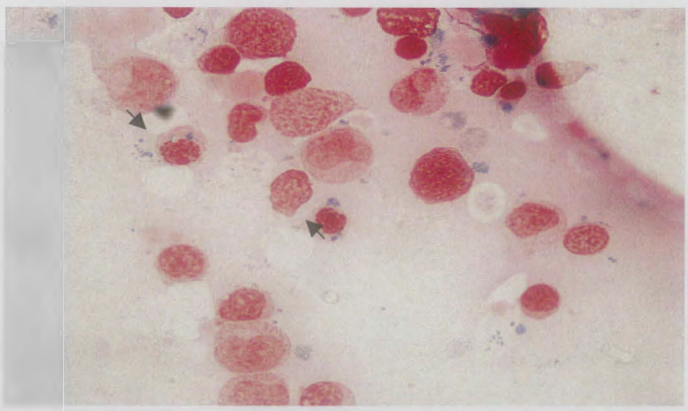

Figure 2. Prussian blue stain of Patient B's bone marrow. Arrows show mitochondrial iron deposits in developing erythroid cells.

Alcohol also affects the absorption of iron directly. This is due to its direct toxic effect on the gastrointestinal tract, causing increased iron absorption. In those alcoholics without blood loss, this may result in acquired haemochromatosis (5). The characteristic mitochondrial iron loading seen in the sideroblastic state is therefore exacerbated [10]. Those alcoholics who develop haemochromatosis are at increased risk for liver cancer.

Folate deficiency

Alcoholics often suffer from both primary and secondary malnutrition. Primary malnutrition is where alcohol replaces nutritious meals (a 'liquid diet'), whereas secondary malnutrition occurs when the alcohol interferes with the absorption or metabolism of important nutrients [6]. One of these nutrients is folate. Folate is an important substrate in haematopoiesis, as it is involved with the synthesis of nuclear DNA. Without enough folate, asynchrony between the nuclear and cytoplasmic development of red cells develop, and cell division is halted. This results in megaloblastic anaemia (7).

The acquired sideroblastic abnormality is only seen in alcoholics with a corresponding folate deficiency. Chronic alcohol ingestion in patients with appropriate folate levels is not associated with a sideroblastic change (1,3). Without a deficiency in folate, a chronic alcoholic will demonstrate macrocytosis and possibly anaemia, but this anaemia will not be sideroblastic.

Originally it was assumed that folate deficiency in alcoholics was solely the result of poor nutrition. However, increased levels of ethanol, as seen in a chronic alcoholic, lead to increased urinary excretion of folate, increased catabolism of folate by the ethanol to acetaldehyde conversion, and malabsorption of folate in the jejunum by inhibiting the enzyme responsible for this. These all lead to a rapid decrease in serum folate levels of as little as 2-4 days (3). Folate deficiency itself leads to a further decrease in serum folate, by causing intestinal folate malabsorption and diarrhoea (8). The severity of folate deficiency is proportional to the amount of alcohol consumed and to the decrease in vitamin intake (8). One nutritious meal a day, however, can prevent the alcoholic from a deficiency in folate (3).

Conclusions

The case of patient B has presented an example of secondary acquired sideroblastic anaemia caused by chronic alcoholism and associated folate deficiency. Through this case and review of recent literature concerning the haematological aspects of chronic alcohol abuse, strong evidence for the requirement of folate deficiency in the development of cases of sideroblastic anaemia caused by chronic alcoholism is found.

The correlation between alcoholism, folate deficiency and secondary acquired sideroblastic anaemia is shown in Figure 3.

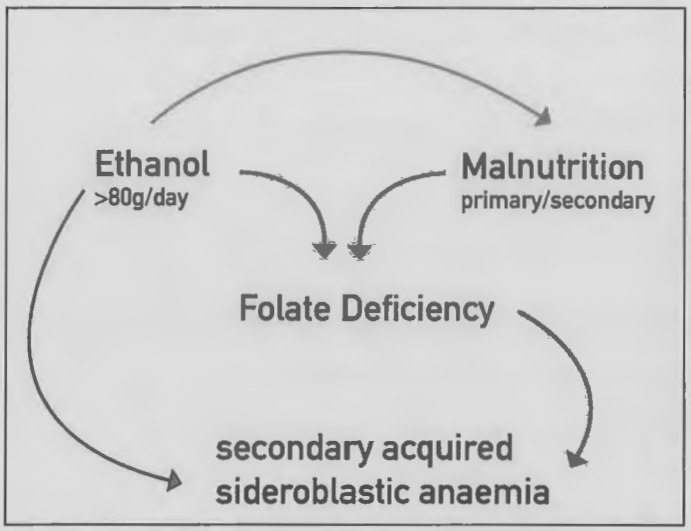

Figure 3. The correlation between chronic ethanol intake, folate deficiency, and the resulting anaemia

The evidence of this correlation is seen in the conclusion of patient B's case – the decrease in ethanol intake below 80 g/day and coinciding folate increase both served to reverse the sideroblastic changes seen in her original presentation.

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References

1 Bottomley S. Sideroblastic anemias. In: Lee GL, Bithell TC, Foerster J, Athens JW, Lukens JN, Eds. Wintrobe's Clinical Hematology. 9th Ed. Philadelphia: Lea & Febiger; 1993.

2 Bridges KR. Sideroblastic anaemias. *Brit J Haematol* 2002; 116: 733-43.

3 Wiley S, Moore MR. Heme biosynthesis and its disorders: porphyrias and sideroblastic anemias. In: Hoffman R, Benz E, Shattil S, Furie B, Cohen H, Silberstein L, McGlave P, Eds. Haematology: Basic Principles and Practice. 4th ed. Philadelphia: Elsevier Churchill Livingstone; 2005.

4 Latvala J, Parkkila S, Niemela O. Excess alcohol consumption is common in patients with cytopenia: studies in blood and bone marrow cells. *Alcohol Clin Exp Res* 2004; 28: 619-24.

5 Ballard HS. The hematological complications of alcoholism. *Alcohol Health Res World* 1997; 21: 42-52.

6 Lieber CS. Nutrition, alcohol use, and liver disease. *Alcohol Res Health* 2003; 27: 220-31.

7 McClintock LA, Fitzsimons EJ. Erythroblast iron metabolism in sideroblastic and sideropenic states. *Hematology* 2002; 7: 189-95.

8 Marsano L. Alcohol and malnutrition. *Alcohol Health Res World* 1993; 17: 284-92.

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Review of molecular methods for medical microbiology testing

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History

Since the conception of the polymerase chain reaction (PCR) in 1983 by Dr Kary Mullis (and others from the Cetus Corporation) and the subsequent publication in *Science* in 1985, this technique has been utilised in many forms in order to produce virtually unlimited copies of genetic material in the laboratory (amplification). Amplification of as little as a single strand of DNA target sequence enables PCR to detect extremely low concentrations of nucleic acid. This technology has reduced the reliance of the clinical microbiology laboratory on culture-based methods and created new opportunities for the clinical laboratory to more efficiently affect patient care (1).

In 1990 Roche Diagnostics purchased the patents related to PCR, imposing license conditions on subsequent users of the methodology. The techniques for these processes have been documented by Roche Diagnostics (2-4).

The use of a range of molecular tools allows highly sensitive and specific, culture-independent detection of infectious agents in clinical specimens (5). An example of the sensitivity obtained by PCR is where *Chamydia* spp. can be detected to a single target in a 10^{12}-fold background of unrelated DNA. An optimised PCR can be at least 150-fold, but more often about 1000-fold more sensitive than the cell culture (6).

This new technology evolved at a time when enzyme immunoassays for viral detection particularly, had reached the limit of their sensitivity (7). Of all fields in medical microbiology, detection by PCR has had the strongest impact on virology where standard microbiological diagnosis by culture requires much greater effort than for many other pathogens (6). For safety reasons molecular techniques, rather than culture, are the most advisable for the detection of highly infectious viruses, e.g. avian influenza (H5N1) and SARS etc.

In the case of medical microbiology, molecular methods also enable the detection of pathogens that are difficult to cultivate, or are slow growing or unculturable (1). Nucleic acids (DNA and RNA) present in infectious organisms can be amplified. The genetic material of many viruses is RNA while DNA is the genetic material in bacteria and other viruses.

Since the original description of PCR, a number of modifications, advances and alternative systems for in-vitro amplification of nucleic acids have been developed to meet various requirements for improved detection of DNA and RNA. Some of these different applications for PCR will be discussed below with reference to both the detection of bacteria and viruses.

The genome of each organism contains highly conserved, coded regions that are common to that type of organism and variable regions that result in individual traits. Primers are usually selected from the highly conserved region of the genetic code specific for the target organism (8).

Principle of amplification

Amplification is based on complementarity between a target nucleic acid sequence of interest, present in infectious agents, and primers specifically designed to bind (hybridise/anneal) only to a region within that target nucleic acid. The complementary nature of the primer-target binding gives the assay specificity (sensitivity is due the amplification of a small number of target sequences as explained below). One strand of DNA runs in the 5 prime (5') to 3 prime (3') direction and the complementary strand runs in the 3' to 5' direction. Primers are designed in the 5' to 3' direction and therefore bind to the two strands of double stranded DNA of the target sequence in opposite directions. Primers are used to initiate new DNA synthesis by heat-stable *Taq* DNA polymerase (derived originally from the hot-spring bacterium *Thermus aquaticus* but now usually produced by recombinant DNA technology.

Nucleic acid primers or probes can distinguish minute differences in genetic sequence. Optimal conditions during amplification allow complementary binding (hybridisation) only if both sequences match exactly. The primers determine the specificity and size of the amplified product. When the primers anneal, *Taq* DNA polymerase, using the DNA building blocks dinucleotide triphosphates (dNTPs) as substrates, initiates replication of the target sequence from the 3' ends of the primers. Repeated cycles of amplification of extracted nucleic acid with specific forward and reverse primers is carried out on a thermocycler resulting in an exponential increase in the initial number of strands of DNA. The exponential amplification of the target sequence gives the PCR assay great sensitivity.

Application of PCR in microbiology

Organisms that are non-viable, slow growing or that cannot be isolated or detected in standard culture systems can all be detected with increased sensitivity and specificity within a suitable time frame by PCR. It enables quantification of the organism as well as detection of antimicrobial resistance in organisms such as methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *Enterococcus* spp. (VRE) (9,10) and *Mycobacterium tuberculosis* (6).

It is imperative that the PCR assay be carefully optimised and validated before introduction into the routine molecular microbiology laboratory. This process should produce a robust assay that has all chemicals at optimal concentrations and the most appropriate thermal reaction parameters. Typically some DNA or RNA sequence of the organism is known (for primer design) otherwise 'universal' primers (e.g. to the 16S region of bacteria) may be used, followed by DNA sequencing to confirm the organism's identity. For amplification of DNA there must be at least one intact copy of the target DNA/RNA present in the sample.

Extraction of DNA/RNA (commercial columns)

Only a small amount of clinical specimen is required. DNA/RNA template quality is very important – especially in the newer forms of PCR that use fluorescent probes. Typically small centrifuge columns containing a DNA/RNA-binding matrix are used in the clinical laboratory rather than the historical organic solvent methods.

The column extraction methods comprise the following basic steps:

Step 1. Lysis

Lysis buffer often containing guanidine thiocyanate or similar plus a detergent such as Triton-X or SDS lyses the cells releasing the nucleic acids. Proteinase K enzyme for protease digestion is also added and rapidly inactivates endogenous nucleases such as RNAses and DNAses.

Step 2. Precipitation

The lysate is then mixed with alcohol to precipitate the DNA and then loaded onto the extraction column.

Step 3. Binding to membrane/washing

DNA is absorbed onto the silica-gel membrane. DNA bound to the membrane is purified by washing with a high salt buffer to ensure complete removal of any residual contaminants such as salts, proteins, cellular components etc.

Step 4. Elution of purified DNA/RNA

Purified DNA/RNA is eluted from the extraction column with a low salt buffer or water. The eluate is then ready for PCR testing with the reaction mix.

Reaction mix components

The reaction mix consists of buffer, Mg^{2+}, dNTPs, primers, *Taq* polymerase enzyme, and water. These components are essential for efficient replication of the target nucleic acid to occur.

Buffer provides the optimal pH and ionic strength for polymerase activity.

$MgCl_2$ forms soluble complexes with dNTPs to produce the actual substrate that the *Taq* polymerase recognises. Mg^{2+} influences enzyme activity and increases the melting temperature (T_m) of double stranded DNA (dsDNA).

dNTPs are required as a supply of individual nucleotides (Cytosine, Adenine, Thymine, Guanine – C, A, T, G) and may be supplied as a sets of four separate deoxynucleotides (dCTP, dATP, dTTP, dGTP) or as a pre-mixed solution. The concentration of $MgCl_2$ and dNTPs must be in the correct ratio for optimum performance since the dNTPs will bind $MgCl_2$.

Oligonucleotide primers contain sequences that are complementary to specific sequences that flank the target sequence of the DNA that is to be amplified. The sequence and concentration of primers determines the overall assay success. Well selected primers increase reaction specificity by ensuring that they bind only to the desired target sequence to be amplified. Stringency of primer hybridisation can be increased by higher annealing temperatures, lower $MgCl_2$ concentrations, optimal concentrations of enzyme and primers, annealing time, extension time and the number of PCR cycles.

***Taq* DNA polymerase** is required to synthesise new dsDNA, beginning at the 3' end of the primer, and facilitates the binding and joining of the complementary nucleotides (*Taq* DNA polymerase does not recognise the end of the target sequence and will continue extending original DNA past the region of interest). Newer *Taq* polymerases (employing "hot start" methods) are thermostable and only activated after the initial denaturation step in PCR cycling at 94°C thereby providing an automatic "hot start" (6). These newer *Taq* polymerases remain inactive during PCR set-up. Hot start enzymes cannot elongate non-specific primer-template hybrids that may form at lower temperatures, which increases sensitivity, specificity and yield while allowing assembly of reactions at room temperature.

Water is included to make up the reaction volume.

Thermocycling

The programmed thermocycler mimics the process of DNA replication. PCR is a three-step process, referred to as a cycle, that is repeated a specified number of times One PCR cycle consists of:

Step 1. Denaturation

The reaction mix is heated to >90°C where all double-stranded DNA is separated into single strands. *Taq* polymerase is not denatured during the high heat denaturation steps.

Step 2. Annealing

The temperature is dropped to between 55-65°C, enabling the forward primer to bind specifically to one target DNA strand and the reverse primer to bind specifically to the complementary target DNA strand. The annealing temperature or Tm varies for different assays and is determined by the melting temperature of the primers and is the temperature at which 50% of the oligonucleotide-target duplexes have formed. ≈

Step 3. Primer extension

The temperature is raised to ≈ 72°C for optimal activity of the *Taq* DNA polymerase enzyme. *Taq* DNA polymerase catalyses primer extension as complementary nucleotides (supplied from the dNTPs) are incorporated, synthesising new dsDNA complementary to the original double stranded target DNA region. *Taq* DNA polymerase can add approximately 60 bases/second at 72°C.

At the end of the first PCR cycle there is double the number of new DNA strands identical to the original target. This new dsDNA then serves as a template for the synthesis of new DNA in the next cycle. With each new cycle the number of synthesised DNA increases exponentially. This is the CHAIN REACTION in the PCR method. This cyclical process is repeated up to ≈ 40 times to amplify the original DNA in an exponential fashion to a product that is easily detectable on a gel (endpoint analysis). Conventional amplification time is ≈ 2-3 hours.

Reverse transcription PCR (RT-PCR)

PCR usually only detects DNA, as RNA is not an efficient substrate for *Taq* DNA polymerase. Therefore, for RNA, it is necessary to perform reverse transcription, using a reverse transcriptase enzyme, before initiating PCR amplification (RT-PCR). (Some enzymes do have both reverse transcriptases and DNA polymerases.) Reverse transcription produces a copy of the RNA strand, as complementary DNA (first strand cDNA) in the presence of Mg^{2+} ions, high concentrations of dNTPs and primers. These primers may be either random (e.g. 6 bases), a stretch of T's, a sequence specific for the organism of interest or a combination of these. The reverse transcription process includes extracted RNA, buffer containing Mg^{2+}, RT enzyme, site specific primer (that selectively primes the RNA of interest), RNAse inhibitor (that disrupts cells and inactivates RNAses) and dNTPs.

The RT-PCR process consists of:

Step 1. Annealing of the primer(s) to the single stranded RNA.

Step 2. RT enzyme catalysing primer extension to create first strand cDNA.

Step 3. The resulting first strand cDNA with PCR premix is then available for amplification.

Nested PCR

Nested PCR has the ability to increase both the sensitivity and specificity of PCR, by using two sets of primers an "external" and an "internal" set and two rounds of amplification. There is an increase in the sensitivity from increased total cycle number. Increased specificity is obtained from annealing of the second inner primer set to sequences only amplified from the first PCR. However one disadvantage of nested PCR is that when the second round amplification is set up there is the potential for contamination (1). A variation of nested PCR is a hemi-nested PCR where the second primer set involves an internal primer and one of the external primers.

Multiplex PCR

PCR assays utilise two or more primer sets in the same reaction, this enables simultaneous co-amplification of different nucleic acid targets. Multiplexing can only work when the same set of amplification conditions are suitable for all primers and the primers don't anneal to each other. Additionally the products formed must differ adequately to enable accurate interpretation of the results. However this method is often less sensitive than PCRs with single primer sets due to competition between targets to be amplified (11).

Internal controls

Controls can be added to the PCR amplification mix to detect inhibition of PCR amplification and as a control for the correct performance of the specimen preparation and amplification steps. Internal controls are used to monitor variability within a PCR assay attributed to differences in reaction components, PCR inhibitors, operator-to-operator variance and instrumentation performance. The absence of amplification of an internal control due to inhibitors could also indicate prevention of amplification of the desired organism, so that false negative results could be obtained. However, if the internal controls are added directly to the PCR mix then the assay is, essentially, a multiplex assay. Alternatively a reference gene that is found in all human nucleated cells such as ß globin can be assayed separately for the presence of inhibitors.

Limitations of PCR

Inhibitors

The presence of inhibitors in clinical specimens such as heme, acidic polysaccharides, nucleases, EDTA, SDS and guanidine HCl have all been demonstrated to inhibit PCR (12), by interfering with the action of DNA polymerase (6). An internal control (e.g. an artificial construct or a reference gene in the sample) should be incorporated into the testing specimen to monitor inhibition of the PCR by such substances that may be present in the patient's specimen (9).

Antimicrobial therapy – where treatment has been successful or alternatively, the patient's own defences may have killed the organism. PCR can still detect non-viable organisms (28) which may no longer be causing infection.

Relevance of result

PCR may be too sensitive for some applications, detecting the nucleic acid of an organism that is present at non-pathogenic levels (13).

Contamination

Cross contamination can easily occur unless a careful work ethic is followed. Stringent adherence to separated work places for the following must be observed:

1. Specimen preparation.
2. Setting up PCR reactions.
3. Post-PCR analysis.

Within each of these work areas the following good laboratory practice should be employed:

1. Keep laboratory areas clean.
2. Use aseptic technique, gloves, dedicated equipment, new/sterile equipment, using positive displacement pipettes with barrier tips, avoid creating aerosols when working.
3. Avoid cross-contamination between samples.
4. Avoid cross-contamination from previous samples.
5. Include control reactions in every run to monitor the test.

A further method that can limit contamination is where the amplicon can be inactivated by incorporating deoxyuridine (dUTP) instead of dTTP in the PCR assay, so that any amplicons that may contaminate subsequent assays are cleaved by uracil-N-glycosylase (9). However this is of limited use unless the assay is established with dUTP i.e before the contamination issue occurs.

Amplicon detection (fragment size analysis) for conventional PCR

Conventional PCR requires end point analysis of the amplified product. Detection of amplicons is by gel electrophoresis to determine fragment size. Electrophoresis refers to the migration of charged molecules through a liquid or gel medium when subjected to an electrical field. Migration rate through a gel matrix depends on several factors – net charge on a molecule at the pH at which the assay is performed, size and shape of molecule, electrical strength of voltage drop, pore size of gel and temperature. The charge in an electrical field moves analytes through the gel (14).

Amplicon detection - components

Agarose gel

The concentration of agarose can be varied, controlling gel pore size to make possible the separation of nucleic acid molecules of a wide range of sizes. Migration of nucleic acid in agarose is affected by buffer used and voltage applied. The higher the concentration of agarose, the smaller the pore size. An agarose gel concentration of 2-4% separates fragments of 25-1000bp (14).

Polyacrylamide gel electrophoresis (PAGE)

For very high resolution of low-molecular weight DNA fragments, polyacrylamide gel is used as the pore size is typically much smaller than agarose (14). An alternative is NuSieve agarose which has higher resolution for visualising small DNA fragments (15).

Loading dye is included with the amplified product to be electrophoresed to increase the density of the sample allowing the amplicon to drop evenly into the well. The loading dye is a small molecule which migrates at a constant rate in an electrical field. This allows the progress of the smallest molecules in the gel to be visualised. The loading dye may be added during PCR reaction assembly or following the amplification process. Unlike ethidium bromide, the loading dye does not actually stain the DNA.

DNA gel stains

Ethidium bromide fluoresces in the presence of ss and ds DNA under long wavelength UV light. However, both the ethidium bromide and the UV light are mutagenic.

SYBR Safe™

SYBR Safe™ binds to DNA and RNA, and was developed for reduced mutagenicity (16). Both the ethidium bromide and the SYBR Safe™ require a UV transilluminator for visualisation of DNA bands.

Molecular weight ladders (MW) are a mixture of DNA fragments, upon electrophoresis result in a regular pattern thus allowing for accurate sizing of DNA bands (amplicon).

EIA (ELISA)

EIA can also be used to detect digoxigenin-labeled amplified products. Products are obtained by using a PCR DIG labelling mix (dNTPs plus digoxigenin-labeled dUTP) in the PCR reaction. The labelled nucleotide is then incorporated into the PCR product as it is formed. The incorporated DIG label is detected using antidigoxigenin-alkaline phosphatase (AP) conjugate and colorimetric or XRay detection. This method increases the detection level where only small amounts of template DNA are available (enabling maximum yield of amplicon), by increasing the sensitivity of the assay by 10-100 fold over gel staining (11).

Table 1. Automated nucleic acid extractors.

Nucleic acid extractor systems	Manufacturer	Website
ABI PRISM™ 6100	Applied BioSystems	www.appliedbiosystems.com/
Biomek® FX^P including PCR setup	Beckman Coulter	www.beckmancoulter.com/products/instrument/automatedsolutions/
NucliSENS® easyMAG ™	Biomerieux	www.biomerieux-usa.com/clinical/nucleicacid/easymag/index.htm
X-Tractor Gene™	Corbett Life Science	www.corbettlifescience.com
AutoGenPrep	GenPrep	www.autogen.com/Products/
Iprep® purification	Invitrogen	https://catalog.invitrogen.com/
Magtration Systems	Precision System Science	www.pss.co.jp/english/products/02.html
Maxwell®16 System	Promega	www.promega.com/maxwell16/default.htm
QIAcube BioRobot MDx MDx DSP workstation	Qiagen	www1.qiagen.com/Products/Automation/
COBAS® Ampliprep® System MagNa Pure LC Instrument	Roche	http://us.labsystems.roche.com/products/molecular/

Table 2. Complete automated molecular detection systems.

Complete molecular systems	Manufacturer	Website
Utilises a mechanised nucleic acid extractor which is teamed with a separate amplification/detection system, such as the - COBAS® Ampliprep®/COBAS® Amplicor™ for PCR, - COBAS® AmpliPrep®/COBAS® TaqMan® System, real-time PCR of up to four simultaneous assays.	Roche	http://www.roche.com/prod_diag_pcr.htm
BioRobot Universal. Automated RT-PCR, PCR, sequencing reaction, gene expression analysis, genotyping and forensic assays.	Qiagen	www1.qiagen.com/products/automation

Table 3. Automated amplification and detection

Amplification and detection system	Manufacturer	Website
GeneAmp7300RT, Prism 7500	Applied Biosystems	https://products.appliedbiosystems.com/
NucleSENS® (NASBA)	Biomerieux	www.biomerieux-usa.com
DNA Engine Opticon	BioRad	www.biorad.com
SmartCycler®	Cepheid	www.cepheid.com
Rotor-Gene™ 6000 RT cycler	Corbett Life Science	www.corbettlifescince.com
Mastercycler® ep *realplex*	Eppendorf	www.eppendorf.com
Rapidcycler® 2	Idaho Technology	www.idahotech.com/rapidcycler/
Cobas® Amplicor® Analyzer (PCR), commercial kits.	Roche	www.roche.com/prod_diag_pcr.htm
Real Time Detection Lightcycler® 2.0 LightCycler 480 real-time PCR System Cobas® Taqman® 48 analyzer for detection of HIV, HBV, HCV nucleic acid and as many as 10 different user-defined PCR profiles Cobas® Taqman® 96 analyzer automated, for the amplification and detection of viruses like HIV, HBV, and HCV.		
Mx300P™	Stratagene	www.stratagene.com

PCR purification (for sequencing)

Amplified DNA binds specifically to glass fibres in the purification spin columns in a similar way to template extraction systems. Bound DNA is purified by washing and spinning to remove contaminating primers, unincorporated nucleotides and salts. Purified DNA is eluted from the column using a low salt solution.

DNA sequencing (automated)

Amplified DNA product is used as a template which, along with suitable oligonucleotide primers and DNA polymerase, generates fragments that differ in length from each other by a single base. Fragments are separated by size, and bases at the end identified, recreating the original sequence. Various approaches are used to generate and detect the fragments.

One example is the Sanger dideoxy chain terminator method utilising dideoxy bases (ddNTPs) mixed with dNTPs. During replication a base is incorporated into a new chain and, if a ddNTP is incorporated, the replication reaction is terminated (17). A separate reaction is set up for each specific nucleotide with individual fluorescent labelled ddNTPs (in a lower ratio than dNTPs). DdNTPs are randomly added by DNA polymerase which in turn terminates elongation as a 3'-OH group is not present. Reaction products are separated by electrophoresis, which separates molecules according to size. The fluorescent base that terminated the PCR reaction is detected and the base identified.

The sequence obtained can be loaded into a Gene Bank e.g. PubMed, and the nearest sequence match obtained. If the appropriate DNA template has been selected, the genotype can be determined by sequencing. Genotyping may give an indication of virulence properties such as infectivity, virulence, antigenic variation and resistance to antiviral agents.

Quantitative PCR

The detection of fluorescence as an endpoint in real time PCR means target DNA present in the initial sample can be quantified. This is especially useful to monitor treatment. A series of standards with known amounts of target are amplified and a standard curve constructed (13).

Real Time PCR

This method utilises reaction components similar to traditional PCR and varies only in the detection process. Real Time PCR is no more sensitive than conventional PCR but is able to produce a result as the amplified product is being formed (18). The analysis of product formation is performed during the log phase of amplification producing a rapid result in a faster time-frame (Real Time) enabling rapid sample turnaround time. Additionally, real time PCR is performed in a closed system thus minimising the risk of contamination.

Real time PCR combines two instruments in one: a PCR thermal cycler and an integrated fluorescence detection device allowing for fluorescent monitoring to detect the formation of the PCR product. Automated amplification, detection and quantification (bacterial and viral loads) within the same closed system, allows for acceleration and increased efficiency of the process. The reaction time can be as short as 30 minutes (9).

For a commonly-found instrument in clinical laboratories, the LightCycler, reactions are performed in glass capillaries suspended in a chamber allowing a high surface-volume ratio of the PCR mix (9). These capillaries are heated by air allowing rapid, uniform heating producing a more rapid result. Fluorescent labelled probes bind to target DNA releasing fluorescence that is detected continuously by optical software. Fluorescence values versus cycle numbers are displayed. The increasing level of fluorescence is proportional to the number of target copies formed during the PCR process. The point at which fluorescence surpasses the noise threshold during amplification is called the threshold cycle or crossing point and is proportional to the number of amplified target copies present in the sample at that time (13).

Fluorescent signal can be detected by a number of systems such as:

- ethidium bromide, SYBR green
- hydrolysis probes, hybridisation probes
- molecular beacons, LUX probes
- scorpion primers
- Minor Groove Binding (MGB, Eclipse) probes.

Ethidium bromide and SYBR green dyes bind (intercalate) to any ds DNA and the increase in fluorescence is measured. However SYBR Green is more sensitive and specific than ethidium bromide (18). See Figure 1.

Sequence-specific probes are labelled with Fluorescence Resonance Energy Transfer (FRET) fluorophores. Fluorescence occurs due to a distance-dependent transfer of energy between two adjacent fluorophores and a fluorescent signal is only produced if the specific target is present. Alternatively, the energy may be transferred from a flourophore to a non-fluorescent compound and dissipated as heat, i.e. quenched, and in this case, fluorescence only occurs when the two compounds are separated (6). Descriptions of each type of probe follow.

Hydrolysis probes (Figure 2) e.g. "TaqMan® probes" (Roche Diagnostics), are labelled with a fluorescent reporter (R)(donor) and quencher (Q)(acceptor) in close proximity to each other. Specific binding to DNA target occurs, when DNA polymerase encounters the non-extendable probe, the probe is digested and R and Q are separated (the reporter is no longer quenched). The reporter can then emit fluorescence upon excitation (10). As TaqMan probes are hydrolysed they cannot be used for melting curve analysis.

Hybridisation probes (Figure 3) e.g. HybProbe™ hybridisation probes (Roche Diagnostics) comprise two fluorescent labelled, target-specific probes (Donor and Acceptor) that anneal to specific ssDNA. In close proximity the probes fluoresce due to FRET (10).

Molecular beacons and Scorpion™ primers also have a fluorescent reporter (R) and quencher (Q) in close proximity to each other. Sequence-specific binding results in an increase in the distance between R and Q to yield a detectable fluorescence (6).

LUX probes LUX™ (Light Upon eXtension) (Invitrogen). LUX™ primer sets include a self-quenched fluorogenic primer and a corresponding unlabeled primer creating a hairpin secondary structure which provides optimal quenching of the fluorophore. When dsDNA binds during PCR, the fluorophore is no longer quenched and fluorescence occurs (20).

Minor Groove Binding (MGB, Eclipse) probes

These dsDNA-binding agents are attached to the 3' end of TaqMan® probes to increase the T_m value (by stabilization of hybridization). This process increases efficiency of reporter quenching (http://dorakmt.tripod.com/genetics/glosrt.html). Probes and primers can be made in many colours encompassing wavelengths from UV to infrared, making multiplex detection available (21).

Melting point analysis can be performed with PCRs utilising hybridisation probes and molecular beacons as they are not hydrolysed by *Taq* polymerases to generate signal. This step occurs immediately after the PCR assay. Melting curve analysis confirms the PCR product as the correct amplification product and can distinguish base pair differences (mutations or polymorphisms in target DNA) (9) e.g. genotyping Herpes simplex virus types 1 and 2 (19) and distinguishing multiple rifampin

resistance mutations and high-level ioniazid resistance mutations in *Mycobacterium tuberculosis* (6).

Analysis is performed by raising the temperature while continuously monitoring the fluorescent signal. SYBR Green melting curve analysis allows monitoring the melting behaviour of the PCR products and allows discrimination between specific product and primer dimers (non-specific binding to form non-specific products). Hybridisation probe melting curves detect the separation of target hybridisation probes. Probes that are bound to perfectly matching target DNA dissociate at a higher T_m than DNA that contains sequence variation.

Molecular methods – other than PCR™ amplification

These methods are based on signal, target, probe or rolling circle amplification.

Signal amplification techniques

The principle of signal amplification is to directly detect nucleic acids without target amplification by increasing the signalling capacity of the labelled molecules attached to the target nucleic acid eg. branched DNA probes (bDNA), Hybrid capture (anti-DNA-RNA hybrid antibody). Advantages of signal amplification methods over target amplification methods are that the system does not require a thermal cycler and the number of target molecules is not altered so the signal generated is directly proportional to the amount of target sequence present in the clinical specimen. These methods can be carried out at a single temperature (isothermal), are not affected by the presence of enzyme inhibitors, so simpler nucleic acid extraction methods can be used and are less susceptible to contamination. The method can directly measure RNA levels thus eliminating the requirement for cDNA synthesis (1).

bDNA probe methods utilise a branched multiple probe-enzyme complex. The primary probe captures the nucleic acid target onto a solid surface. Branched probes specific for the primary probes are added followed by enzyme-labelled probes which are detected by chemiluminescent substrates to produce a signal which can be quantitated.

An example of Hybrid Capture is the Digene HPV test. The HC2 High-Risk HPV DNA test uses Hybrid Capture 2 technology, a nucleic acid hybridisation assay utilising microplate chemiluminescent detection. Specific HPV RNA probes hybridise to target DNA. The resultant RNA: DNA hybrids are captured onto a microwell solid surface which is coated with antibodies specific for the hybrids. Alkaline phosphatase conjugated specific antibodies bind to the hybrids, and are detected by a chemiluminescent substrate. Multiple conjugated antibodies bind to each capture hybrid resulting in substantial signal amplification (22).

Target amplification systems (other than PCR)

These methods are similar to the PCR process as they use enzyme mediated processes and oligonucleotide primers that bind to complementary sequences on opposite strands of double stranded targets to synthesise copies of target nucleic acid (1). Detection is by hybridization probes, molecular beacons or chemiluminescent probes.

Transcription amplification methods

Isothermal based nucleic acid amplification methods e.g. nucleic acid sequence-based amplification (NASBA) and transcription-mediated amplification (TMA). These methods do not require a thermal cycler, have rapid kinetics, produce a result in a very short time frame and can be done in a single tube. The disadvantages are poor performance with DNA targets and the stability of complex multi-enzyme systems has been questioned (1).

An example of NASBA is the automated NucliSENS EasyQ system (BioMerieux). This system combines two technologies of amplification and detection. In an initial enzymatic amplification of nucleic acid target, single-stranded RNAs are generated. Detection occurs with exponential amplification of the target sequence when specific molecular beacon probes hybridize causing fluorescence (23). NASBA is able to specifically amplify and directly detect RNA target in a DNA background and can detect RNA without DNA contamination. RNA amplicons are single stranded and therefore do not require denaturation prior to detection.

An example of a TMA based assay is the Gen-Probe kit for the detection of *Chlamydia trachomatis*. The method involves a promoter primer that binds to the rRNA target. Reverse transcription creates cDNA. A second primer binds to cDNA and reverse transcription creates a new DNA copy. RNA is transcribed from the DNA template to produce many copies of target.

Strand displacement amplification (SDA)

Isothermal nucleic acid amplification of a particular sequence by a two step process - target generation and exponential target amplification. The primer containing a restriction site binds to the target and DNA is synthesised. The restriction enzyme nicks the strand containing the restriction site and polymerase begins amplification again, displacing the newly synthesised strand. This method has the advantage of being technically simple to perform (1).

An example of SDA based assay is the BD ProbeTec™ assay for the detection of *Chlamydia trachomatis and Neisseria gonorrhoea* using simultaneous amplification of nucleic acids by SDA and real-time detection by using fluorescence resonance energy transfer (FRET) in a one hour format.

Probe amplification techniques

Probe amplification methods amplify products containing only the probe sequence. Some examples are Ligase Chain Reaction (LCR), Cleavase-Invader assays and Cycling Probe technology.

Ligase chain reaction (LCR)

The LCR differs from PCR because it ligates the probe molecule. Two probes are used for each DNA strand and are joined (ligated) together to form a single probe. Amplification is by thermal cycler and, since both the ligated product and the original strand can act as a template there is an exponential increase in product. To improve specificity, LCR commonly uses two enzymes, both a DNA polymerase enzyme and a DNA ligase enzyme, to drive the reaction so LCR can have greater specificity than PCR. For this gapped LCR (G-LCR), a short gap is left between the two annealed probes and is filled by the DNA polymerase before the remaining "nick" is filled (ligated) by DNA ligase, amplification will only occur if the probe has been ligated (1). An example of LCR – probe amplification, was the Abbott detection kit for *C. trachomatis and N. gonorrhoea*, but it is no longer commercially available.

Cleavase-invader technology

Invader assays (Third Wave Technology), invader probe amplification (IPA), (www.twt.com/invader_chemistry/invaderchem.htm) is a probe amplification system that detects nucleic acid target using specific probes, one of which overlaps. The overlapping probe region (flap) is cleaved by a DNA polymerase called Cleavase. The resulting cleaved molecule is amplified and serves as a template for a second reaction. The flap binds to a fluorescent labelled probe releasing fluorescence (FRET). Repeating the process amplifies the signal.

Cycling probe technology

Cycling probe technology (CPT; ID Biomedical Corp., Bothell, Washington) (http://bio.takara.co.jp/bio_en/catalog_d.asp?c_ID=C1265) employs a DNA-RNA-DNA fluorescent labelled probe at a constant temperature. The probe labelled with fluorescence one end and a Quencher the other, anneals to target DNA. An enzyme cuts the RNA region of the probe, the probe is no longer intact unquenching the signal, resulting

in emission of fluorescence. Probe amplification is linear and not exponential. This application has been used to detect the *mec*A gene of MRSA. This method can be used for quantitative (1).

Rolling circle amplification (RCA)

Isothermal amplification, distinct from other isothermal signal, target or probe amplification. A DNA probe anneals to target DNA, the probe acts as a primer for RCA. The free end of the probe anneals to a small circular DNA template. DNA polymerase extends the primer generating linear strands of DNA consisting of multiple repeated copies that can be readily detected. Advantages are that the method is simple, robust, has good sensitivity and is able to be multiplexed.

Automated robotic molecular platforms

Automation of the molecular microbiology laboratory has the potential to assist streamlining of testing processes by offering reliability and standardisation, particularly in high-throughput laboratories. This is accomplished by removing the possibility of operator error and variation. Laboratory personal are also better protected from overuse syndrome due to performance of an excessive number of manual manipulations. Automated equipment can be purchased to perform one part of the PCR process, several or all of the steps required to obtain a result. Some automation systems only operate with pre-packaged applications.

Automated nucleic acid extractors

There is a wide range of DNA and/or RNA extractors available from various companies, capable of handling as few as six and up to several hundred samples per day. Samples that can be processed range from whole blood, serum, plasma, tissue, urine to aspirates such as CSF and sputum. Some extractors are designed to only process limited sample types. Some examples of nucleic acid extractors are listed in Table 1.

Automated PCR setup

Separate machines can also be purchased to prepare and set up PCR reactions.

Full system from extraction, amplification to detection

Complete performance of the molecular detection of microbiology pathogens can be performed by several automated systems. All that is required is the loading of the samples by laboratory personnel. Some examples are listed in Table 2.

Automated amplification and detection

There is a wide range of automated PCR amplification and detection systems available. However, some systems can only detect pathogens using the appropriate commercial kit. Some examples of automated amplification and detection systems are listed in Table 3.

A comparison of some of these systems can be viewed at

http://www.biocompare.com/matrix/2838/Real-Time-PCR-Thermal-Cyclers--Thermocyclers.html

Emerging DNA-based detection technologies

TM BioScience multiplex PCR – Luminex Technology

Luminex Technology utilises microspheres which are tiny color-codes beads that are divided into 100 distinct sets. Each bead set can be coated with a reagent specific to a particular bioassay of interest e.g. an oligonucleotide probe, allowing the capture and detection of specific microbiological pathogens from a sample. Within the Luminex analyzer, lasers excite the internal dyes that identify each microsphere bead, as well as any reporter dye captured during the assay. Many readings are made on each bead set, further validating the results. In this way, Luminex technology allows multiplexing of up to 100 unique assays within a single sample rapidly with sensitivity and specificity (24).

What next?

Lightcycler® SeptiFast test

This test is currently available for the detection and identification of bacterial and fungal DNA. Using a 3 ml whole blood sample, that does not require prior incubation or culture steps and producing a result in less than 6 hours. The LightCycler® Septi*Fast* Kit detects and identifies the 25 most important pathogens known to cause blood stream infections (25).

SmartSense™ is a bio-electronic technology that has been developed by BioWarn, LLC, which allows the real-time detection and identification of pathogens. The SmartSense™ sensor is based on a semiconductor chip to which a ligand (antibody) has been bound. When the ligand combines with its target (antigen), an electrochemical signal is sensed by the chip, amplified and processed to obtain pathogen identification. This technology enables low-cost, hand-held, highly sensitive robust detection capability. Since the system is semiconductor chip-based, the detector system can be reduced to very small size and low unit cost. SmartSense™ prototypes have been fabricated, laboratory tested, and have potential applications for H5N1 virus, MRSA, HIV, opportunistic respiratory infections and VRE (26,27).

MicroArray technology may use a PCR amplified product. This step minimises the amount of specimen required from a clinical sample as well as increasing the sensitivity of the assay. This product binds specifically (hybridises) to probes of interest present on a DNA hybridisation chip. Many probes can be present on the microchip enabling multiple screening in the one test. This type of testing is particularly useful for emerging pathogens requiring multiplexing assays where the typing and sub-typing of the organism can be obtained in the one test. This application has the potential to be miniaturised, enabling bedside testing (hand-held gene analysers), and thus will alter the future implementation and application of DNA diagnostic assays. Currently the cost of this methodology limits its use in the laboratory.

Bio-defense detection

With the emergence of bio-terrorism rapid, hands- free, closed systems (to avoid contamination or carryover) that can be used in the field are an important area that is being developed for the detection of organisms such as anthrax, plague, smallpox, ricin toxin, Staphylococcal enterotoxin-B and avian flu (27). Molecular detection is also required for possible agents of bio-terrorism to provide rapid discrimination of weaponised pathogens from harmless laboratory-adapted or vaccine-related strains (13). An example is the use of Real time PCR to provide rapid screening for the presence of *Bacillus anthracis* spores (6).

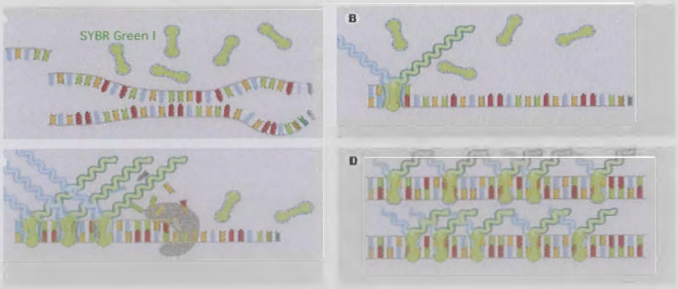
The molecular world is constantly developing and has huge potential. Current methods can be improved to decrease detection time and further enhance specificity and sensitivity. As PCR cannot satisfy all the molecular needs of the microbiology laboratory, non-PCR amplification methods are being developed further. Molecular detection is a powerful tool for the detection of infectious agents in the medical microbiology laboratory and advances in this field will greatly enhance patient care.

Molecular testing technologies currently available in New Zealand can be viewed in Table 4.

Acknowledgement

I wish to thank John Mackay, molecular biologist, Linneaus Limited, Gisborne for his expert assistance in the development of this manuscript.

SYBR Green / Overview



Roche Diagnostics NZ Education Centre

Figure 1. SYBR green probes.

A. Denaturation. Single stranded DNA, primers and SYBR green probes.

B. Annealing. Primers bind.

C. Extension. *Taq* polymerase anneals at the primer site and extends the primers to form cDNA and SYBR green probes bind to any dsDNA, giving off fluorescence.

D. Cycle completed, producing a copy of the target sequence.

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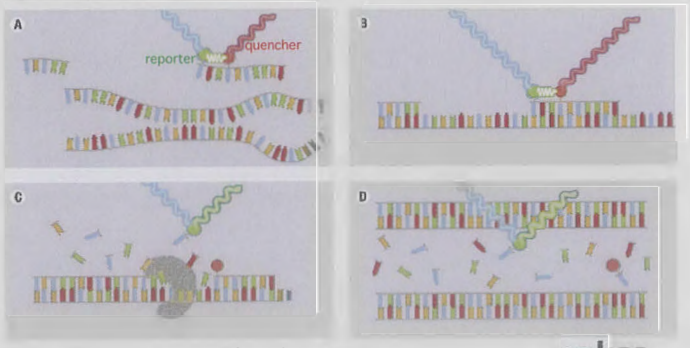
Hydrolysis Probe Overview



Roche Diagnostics NZ Education Centre

Figure 2. Hydrolysis ('TaqMan®') probes.

A. Denaturation. Single stranded DNA, primers and single target specific fluorescent labelled probe.

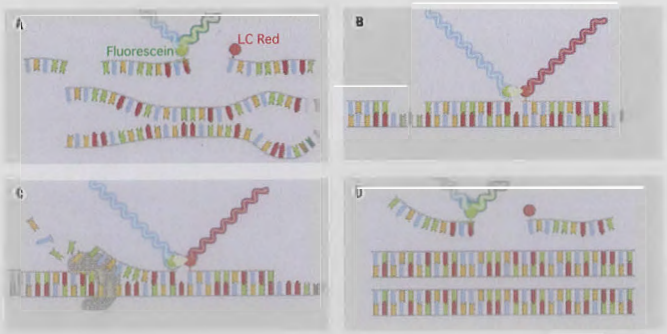
B. Annealing. Primers and target specific probe bind.

C. Extension. *Taq* polymerase anneals at the primer site and extends the primers to form cDNA and in the process "overwrites" the probe, breaking it into separate nucleotides (hydrolysis), separating the reporter and quencher resulting in fluorescence.

D. Cycle completed, producing a copy of the target sequence.

Picture reproduced by permission of Roche Diagnostics, New Zealand.

Hybridization Probe Overview



Roche Diagnostics NZ Education Centre

Figure 3. HybProbe™ hybridization probes.

A. Denaturation. Single stranded DNA, primers and two target specific fluorescent labelled probes.

B. Annealing. Primers bind and both labelled probes bind in close proximity, LC Red 640 emits a signal (FRET) only when both labelled probes are hybridized to the target sequence.

C. Extension. *Taq* polymerase anneals at the primer site and extends the primers to form cDNA, releasing and separating the probes.

D. Cycle completed, producing a copy of the target sequence.

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Pathogen	Testing laboratory and method of detection (where available)	Sample type	Notes/comments
Adenovirus	A+CHL[2,8]	Respiratory – nasopharyngeal swab or aspirate, sputum. Urine (A+, CCHL). Faeces, tissue biopsy (A+).	Alternative – cell culture, method of choice (CHL). Red eye, pneumonia, hepatitis, encephalitis, haemorrhagic cystitis, gastroenteritis.
Aspergillus spp.	CHL[2]	EDTA blood.	Alternative - culture, slow growing and requiring specialised culture media.Fungal PCR.Assay is specific for pathogenic *Aspergillus* spp.
Atypical *Mycobacterium* spp. (refer *Mycobacterium tuberculosis*)			
Avian Influenza	A+[1] ESR[3] CCHL CHL[3]	Respiratory – nasopharyngeal aspirate (NPA), pernasal swab, sputum.	Bird flu.Not cultured due to highly infectious nature of specimen. H5N1 only (CCHL).
Bartonella henselae (Cat Scratch Disease)	A+[8]	EDTA or CPD blood, tissue.	Alternative - culture, slow growing and requiring specialised culture media; formerly named *Rochalimea henselae*.
BK virus	A+CHL	Urine and EDTA blood, cerebospinal fluid (CSF).	Polyoma virus.Haemorrhagic cystitis.
Bordetella pertussis	A+, Waikato CCHL[1] CHL[3]	Dry nasopharyngeal swab.	Alternative – culture, slow growing and requiring specialised culture media. Pertussis, whooping cough. PCR remains positive longer than culture. Confirmation and typing (ESR).
Chlamydia trachomatis	A+ [5] Waikato[6] CCHL[4] APATH[6] CHL[5] Various other laboratories	First catch urine (FCU), endocervix/ urethral swabs. Eye swab (CCHL).	Alternative – cell culture, slow growing and requiring specialised cell culture. Lymphogranuloma venereum (LGV) typing (APATH).
CJD	Australian CJD registry, Melbourne University	CSF 0.5 ml, 20 mL EDTA whole blood.	Specimens forwarded from CHL. Creutzfeldt-Jakob disease, 14-3-3 protein, prion protein gene, PRNP, spongiform encephalopathy. CJD polymorphism risk factor codon 129.
Coronavirus (refer human coronavirus)			SARS, human coronavirus. Common cold.
Coxsackie virus (refer Enterovirus)			Hand foot and mouth disease.
Cytomegalovirus (CMV)	A+, CCHL[1] CHL[1,2]	EDTA blood, tissue, CSF, bronchoalveolar lavage (BAL), urine, amniotic fluid, respiratory.	Alternative – cell culture of tissue and urine, slow growing virus. Qualitative and quantitative PCR. CMV drug resistance testing, EDTA blood (A+).
Echovirus (refer Enterovirus)			
Enterovirus – Coxsackie A and B, Echovirus, Enterovirua, Poliovirus	A+ Waikato[1] CCHL[1] ESR CHL[2]	CSF.Acute phase serum (A+).	Alternative – cell culture, slow growing virus, samples sites vesicle fluid, throat, and rectum. Culture enables typing of virus. CSF culture not as successful as PCR detection (low viral load). Polio typing PCR/ELISA from culture isolate (ESR).
Epstein Barr virus (EBV)	A+[1]	CSF, fresh tissue A+.EDTA blood, unseparated, stored at room temperature, CCHL.	Infectious mononucleosis, glandular fever. Quantitative PCR.
Hepatitis B (HBV)	A+ ESR[6]	Serum, liver biopsy.	Quantitative. HBV YMDD mutation, serum (A+).

Pathogen	Testing laboratory and method of detection (where available)	Sample type	Notes/comments
Hepatitis C (HCV)	A+ [4] CCHL [4] (qual. only) ESR CHL [1] (qual.& genotyping only)	Serum, liver biopsy.	HCV qualitative, quantitative and genotyping,
Hepatitis D (HDV)	A+ [6]	Serum, liver biopsy.	HDV
Herpes simplex virus (HSV 1 and 2)	A+ Waikato [1] CCHL [1] APATH [1] CHL [1, 2]	CSF, tissue, lesion swab, BAL.	Alternative cell culture, DFA (where indicated) Typing by Real time PCR using meltback analysis for virus typing. In house PCR, typing by restriction endonuclease (RE) digestion for CSF (CHL)
Herpes zoster virus (refer varicella zoster)			HZV
Human coronavirus	CHL [3,8]	Respiratory – nasopharyngeal swab (NPS), lung, sputum.	(not SARS) HCV 229E, HCVOC43
Human herpes virus type 6 (HHV6)	A+ CHL1	EDTA blood, CSF.	Roseola infantum, xanthema subitum, Sixth disease.
Human herpes virus type 8 (HHV8)	A+	EDTA blood, serum, biopsy. Karposi sarcoma, bone marrow (BM).	Considered a necessary prerequisite for Karposi's sarcoma. Association with a number of conditions including multicentric Castleman's Disease, multiple myeloma and in primary effusion lymphomas (A+).
HIV	A+ Waikato CCHL [4] CHL [4] (HIV VL only)	Qualitative 1 x EDTA blood. Quantitative 2 x EDTA blood.	Human immunodeficiency virus, AIDS, retroviral illness. HIV viral load (VL). Qualitative, quantitative PCR. Pro-viral HIV DNA (HIV1 and HIV2) (A+). HIV genotype resistance testing (A+).
HIV drug resistance genotying	A+	1 x EDTA blood.	HIV resistance testing.
Human papilloma virus (HPV)	A+ Waikato [7]	Tissue, biopsy, genital swab, scraping, cytobrush.	HPV cannot be cultured. Genital warts, papilloma virus
Influenza A, B	A+ [1] ESR [3] CHL [3]	Respiratory – NPA, pernasal, sputum.	Alternative, DFA, Rapid testing, cell culture.A+ influenza A only. Refer Avian flu separately.
JC virus	A+ CHL	CSF, urine.	Polyoma virus. Jamestown Canyon virus.
Legionella spp.	Waikato ESR CHL [2,8]	Serum, urine, sputum, BAL.	Alternative culture, slow growing fastidious bacteria, requiring specialised media, recovery rate low. Sequencing for speciation (ESR, CHL).
Leptospira spp.	ESR CHL [3]	Acute – CSF, serum/EDTA plasma. Convalescent – urine, tissue.	Alternative culture, slow growing spirochaete and requiring specialised media.
Measles virus	A+ CHL [3]	CSF, EDTA blood/serum, fresh brain biopsy, NPS.	Morbillivirus, English measles.
Meningococcal PCR – refer *Neisseria meningitis* PCR			
Metapneumovirus	CHL [1,8]	Respiratory – NPS, sputum.	Non culturable. Non routine.
Mycobacterium tuberculosis (TB)	A+ [8] CHL [4]	CSF, tissue, pleural or pericardial fluid, bone marrow, fine needle aspirate (FNA). Paraffin embedded tissue (A+).	Alternative culture - slow growing bacteria and requiring specialised media. *M. tuberculosis, M. bovis, M. bovis* (BCG). HSP65 for *Mycobacterium* spp. *M. tuberculosis, M. avium*-complex and *M. gordonae* by Rapid DNA probes (A+), *M. tuberculosis, M. avium and M. intracellularae* (CHL).
Mycoplasma genitalium	APATH [1]	Genital swab.	Multiplex PCR with *T. vaginalis*, *U.urealyticum* and *U. parvum* also detected.
Mycoplasma pneumoniae	CHL [2]	Throat swab, sputum, BAL.	Alternative culture - slow growing bacteria and requiring specialised media.

Pathogen	Testing laboratory and method of detection (where available)	Sample type	Notes/comments
Neisseria meningitidis	A+ Waikato CCHL[1] ESR CHL[1]	CSF, EDTA blood. Fresh tissue (CCHL).	Alternative bacterial culture. Meningococcal meningitis. Characterisation by sequencing (ESR).
Norovirus	CHL[2] ESR[2] (typing)	Faeces, vomit (sometimes if no faeces available).	Nonculturable. Calicivirus, Norwalk-like virus (NLV), small round-structured virus (SRSV). Genotyping by DNA sequencing (ESR).
Papilloma virus (HPV) refer human papilloma virus			
Parainfluenza viruses 1,2,3	CHL[3,8]	Respiratory – NPS , BAL.	Alternative cell culture.
Parvovirus B19	A+ CHL[3]	Serum, bone marrow in EDTA, throat swab (TS), amniotic fluid, foetal blood, post mortem tissue, CSF, joint aspirate.	Erythrovirus B19. Erythema infectiosum (fifth disease, "slapped cheek syndrome").
Picornavirus (Enterovirus and Rhinovirus)	CHL[3,8]	Respiratory – NPS, sputum.	Alternative cell culture, slow growing virus.
Polio (refer Enterovirus)			Acute flaccid paralysis, AFP.
Respiratory Syncytial Virus	CHL[3,8]	Respiratory – NPS, sputum.	Alternative cell culture. RSV
Rhinovirus (refer Picornavirus)			Common cold.
Rickettsia spp.	A+[8]	EDTA blood	Typhus Fever group Spotted Fever group Tsutsugamushi Fever group Culture difficult and potentially dangerous.
Rubella	A+[1]	CSF, EDTA blood, fresh placental biopsy, fetal blood.	German measles.
SARS (coronavirus)	A+ CCHL[1] CHL[1,3,8]	Respiratory – NPS, sputum, lung. Faeces, serum/EDTA plasma, CSF.	Severe Acute Respiratory Syndrome. SARS-Cov, Urbani virus, HcoV.
Streptococcus pneumoniae	ESR CCHL	CSF, PM samples. EDTA blood (CCHL, ESR) Tissue aspirates (ESR).	
Toxoplasma gondii	A+ CHL[1]	Acute – CSF, EDTA blood, tissue. Intrauterine infection – amniotic fluid (AF), fetal blood (EDTA). Congenital infection – AF, placental biopsy, cord blood (EDTA).	Toxoplasmosis, protozoan parasite.
Trichomonas vaginalis	APATH[1]	Genital swab.	Trich. Multiplex PCR with M. genitalium, U.urealyticum and U. parvum also detected.
Tropheryma whipplei	A+	CSF, fresh tissue.	Whipple's disease. *T. whippelei. Tropheryma whipplei* culture difficult and insensitive.
Ureaplasma parvum	APATH[1]	Genital swab.	Multiplex PCR with *M. genitalium, T. vaginalis* and U.*urealyticum* also detected.
Ureaplasma urealyticum	APATH[1]	Genital swab.	Multiplex PCR with *M. genitalium, T. vaginalis* and *U. parvum* also detected.
Varicella zoster (VZ)	A+ Waikato CCHL[1] CHL[1,2]	CSF, EDTA blood, (A+, CCHL), tissue, skin/genital swab.	Alternative – culture, difficult to grow. Chicken pox, shingles, VZV, VZ, HZV, herpes zoster, zoster. CSF In house PCR (CHL).
Whipple's disease (refer *Tropheryma whipplei*			
Additionally Bacterial species	A+[8]	Any sample where organisms are seen on Gram stain.	Universal primers for 16S rRNA amplification and sequencing. For speciation to genus level (and sometimes to species level). The test is for guidance only and in conjunction with clinical consultation.

Molecular detection method:

1. Real time PCR
2. PCR in house
3. PCR in house nested
4. Cobas Amplicor (PCR and ELISA)
5. SDA - Strand displacement amplification
6. Real time PCR (Cobas Taqman)
7. Nucleic acid hybridisation with signal amplification (Digene)
8. Requires consultation with the Microbiologist

References for testing laboratories:

A+ - Labplus, Auckland City Hospital, Auckland. Laboratory Handbook 2007. www.labplus.co.nz/microbiology.htm .

ESR – Environmental Science & Research, Wellington. www.esr.cri.nz/SearchResults.aspx?query=pcr

Waikato – Waikato Laboratories, Hamilton. www.waikatodhb.govt.nz/laboratory/molecular_biology.htm.

CCHL - Capital Coast Health Laboratory Wellington Hospital, Wellington. www.ccdhb.org.nz/HHS/lab/GenericList.asp.

APATH - Aotea Pathology, Wellington. Dr Collette Bromhead, personal communication (0800 500430).

CHL – Canterbury Health Laboratories, Christchurch. www.cdhb.govt.nz/chlabs/testsframe.htm.

References

1. Nolte FS, Caliendo AM.. Manual of Clinical Microbiology (8th ed). Murray et al., (eds). ASM Press, USA. 2003. Ch 17, pp 234-256.
2. Roche. Polymerase Chain Reaction. Roche Diagnostics GmbH, Germany. 1998.
3. Roche.Roche Molecular Biochemicals PCR Applications Manual. 2nd edition. Roche Diagnostics GmbH, Germany. 1999.
4. Roche. The Evolution of PCR. Roche Diagnostics GmbH, Germany. 2003.
5. Becker K. Principles and role of nucleic acid amplification and modern microbiological diagnosis (Article in German). *Anasthesiol Intensivemed Notfallmed Schmerzther* 2000; 35: 35-40.
6. Kaltenboeck B, Wang C. Advances in real-time PCR: application to clinical laboratory diagnostics. *Adv Clin Chem* 2005; 40: 219-59.
7. Richman DD, Cleveland PH, Redfield DC, Oxman MN, Wahl GM. Rapid viral diagnosis. *J Infect Dis* 1984; 149: 298-310.
8. Brunk CF, Avaniss-Aghajani E, Brunk CA. A computer analysis of primer and probe hybridization potential with bacterial small-subunit rRNA sequences. *Appl Environ Microbiol* 1996; 62: 872-9.
9. Cockerill FR 3rd. Application of rapid-cycle real-time polymerase chain reaction for diagnostic testing in the clinical microbiology laboratory. *Arch Pathol Lab Med* 2003; 127: 1112-20.
10. Mackay IM. Real-time PCR in the microbiology laboratory. *Clin Microbiol Infect* 2004; 10: 190-212.
11. Syrmis MW, Whiley DM, Thomas M, Mackay IM, Williamson J, Siebert DJ, et al. A sensitive, specific, and cost-effective multiplex reverse transcriptase-PCR assay for the detection of seven common respiratory viruses in respiratory samples. *J Mol Diagn* 2004; 6: 125-31.
12. Al-Soud WA, Rådström P. Purification and characterisation of PCR-inhibitory components in blood cells. *J Clin Microbiol* 2001; 39: 485-93.
13. Normandin M, Tsongalis GJ. Detection methods for nucleic acid amplification products. In: Laboratory Diagnosis of Viral Infections. EH Lennette, TF Smith (Eds.). Marcel Dekker, Inc, New York. USA. 1999. Ch 8.
14. http://www.cambrex.com/Content/bioscience/CatNav.asp?oid=728&prodoid=NusieveGTG
15. Molecular Probes. SYBER Safe™ DNA Gel stain. Molecular Probes, Inc. USA. 2004.
16. Sanger F, Nicklen S, Coulsen AR. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci* U S A. 1977; 74: 5463-7.
17. Mackay J, Landt O. Real-time PCR fluorescent chemistries. *Methods Mol Biol* 2007; 353: 237-61.
18. Espy MJ, Uhl JR, Sloan LM, Buckwalter SP, Jones MF, Vetter EA, et al. Real-time PCR in clinical microbiology: applications for routine laboratory testing. *Clin Microbiol Rev* 2006; 19: 165-256.
19. Evans GE, Beynon KA, Chua A, Anderson TP Werno A, Jennings LC, et al. Comparison of real-time PCR with culture and EIA for the diagnosis of mucocutaneous infection with herpes simplex virus. *N Z J Med Lab Sci* 2006; 60: 92-6.
20. http://dorakmt.tripod.com/genetics/glosrt.html
21. http://www.allelogic.com
22. http://www.digene.com/labs/labs_hpv_01.html
23. http://www.biomerieux-usa.com/clinical/nucleicacid/easyq/easyq_technology.htm
24. http://www.luminexcorp.com/technology/index.html
25. http://www.roche.com/prod_diag_lc-septif.html
26. Riggs JL. New developments in identification of microorganisms and chemicals. 9th International Conference, Detection Technologies, 2006, San Diego, California, USA.
27. http://www.biowarnllc.com

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TH Pullar Address

Stairway to science

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

Firstly I would like to thank Council for the honor and privilege of presenting this 40th year anniversary TH Pullar address.

It was first started in 1967 to acknowledge at each annual scientific meeting the contribution that Thomas Henry Pullar had made to our Institute. Australians in the audience may not be aware of Thos Pullar as he was affectionately known. He was born here in Auckland in 1907, but later his family returned to Edinburgh Scotland where Thos eventually followed his father's footsteps into medicine. He graduated in 1929 and started his professional career as a biochemist to the Sheffield Royal Hospital. In 1937 he moved to New Zealand taking up a position as pathologist at Palmerston North Hospital, a position he held for 25 years. In those days, and likewise today, medical scientists and technicians were the backroom staff and Thos championed and did much to promote them on their stairway to science. He helped to lay down the foundations for medical laboratory science throughout the country, involving himself with the training and welfare of all staff. He drafted a number of conditions of employment as well as setting up syllabi for the Ministry of Health's laboratory discipline examinations.

In 1963 his worsening health demanded a lighter workload and he moved to Tauranga and worked part time at Tauranga Medlab. Thos Pullar died on the 29 August 1966

During January 1965 I started my Stairway to Heaven when I started as a trainee at Hamilton Medical laboratory. This was a very interesting time in the Industry with several new technologies being released which had world wide application.

Rosalyn Yalow, in a paper accepted for publication in March 1960, described a new accurate and elegant methodology "Immunoassay of endogenous plasma insulin in man". While chemical methods for analysis of hormones in blood & urine were in common use in the 1950's, specific analytical procedures were not available for peptide hormones mainly because of their occurrence in blood in extremely low concentrations. She had been working in the area of diabetes research for some time and found that diabetic patients who had received injections of the polypeptide hormone insulin had developed antibodies against the hormone. Now, this conflicted against the prevailing concept at that time, the understanding was that such a small protein as insulin could not be antigenic and for sometime her articles had not been accepted for publication. Rosalyn had to argue with the establishment for quite some time before her article would be published.

The insulin antibodies formed a soluble complex with added insulin labeled with radioactive iodine, and furthermore, when non-labeled insulin was added to this mixture it could displace the labeled insulin bound to the antibody. This may be expressed in another way: the percentage binding of labeled insulin to the antibodies is a function of the total insulin concentration in the solution.

This was to become the starting point for determination of insulin and, later, for all peptide hormones in blood, other fluids and tissues. In a series of brilliant, now classical papers, she described the RIA in detail. It was accomplished by a spectacular combination of immunology, isotope research, mathematics and physics.

Also at this time there was another significant paper in Clinica Chimica Acta Vol 5, Issue 4, July 1960, p453-459 on "The estimation of thyroxine in human plasma by an electrophoretic technique" by Roger P Ekins. Here Professor Ekins relied upon the quantitative dependence of the distribution of thyroxine between albumin & thyroxine binding globulin when exogenous radiolabelled thyroxine is added to serum in vitro.

Today, we have at our disposal a large number of RIA-like procedures, the so-called ligand methods for determination of anything you wish to measure enzymes, viruses, antibodies, drugs etc etc. As an example of this versatility the Los Angeles Times reported in September 2005 in their art section, about a painting from the 1600 that was hanging in the Getty museum and the debate that was going on in the art circles about what material had been used to produce the skin colour of the mythical Greek figures. A small piece of the artwork had been taken and using an ELIZA method and it had been identified as egg white.

In 1957 Leonard Skeggs invented the autoanalyser in his home basement and it's widespread introduction during the 1960's resulted in an exponential increase of testing, and forced a radical and profound change on the organisation of laboratories. Previously the number of tests were restricted because of limitations of staff availability and inclination. The autoanalyser with it's 40 tests/hour opened up the prospect of test volume being limited only by the number of analysers. Development moved from single test analysers to 6, 12 and SMAC which could produce 20 test results every 20 secs.

As time went by control and discretion were removed from the staff and placed directly onto the analysers. For example, haematology departments screened all blood films manually in those days compared with now where modern analysers screen up to 80%.

This mechanization of work forced changes on the laboratory organisation, almost simultaneously with the advent of autoanalysers was the introduction of a new work group, the laboratory assistant. Increasingly analytical functions were done by one or two machines with relatively unskilled staff feeding the samples in.

Most technologists took to the autoanalysers with enthusiasm and used their expertise to convert manual methods onto the systems. Some technologists transferred from the traditional laboratories to work in the medical supply companies to create new systems and develop reagent kit sets.

Kit sets were a major change at that time. Some of us still practicing our craft in 2007 can recall having to make up most, if not all, of their own reagents and primary standard before starting a test procedure. Most departments had an extensive chemical store to prepare simple stable reagents. However, once this kitset industry got under way, it grew rapidly to cover a wide menu of tests including enzyme assays, antibodies, specific proteins, and RIA.

The kit sets contained all the calibrators, QC samples and reagents necessary for performing batches of tests in a single package. Specialised staff were no longer required to prepare complex reagents, they could be bought in a kitset and in combination with analysers were producing improved analytical precision. Procedures that previously required hierarchical specialist departments were centralized and new names were being heard, Core Lab, Immunochemistry, BiochImm and Halls of Automation.

In the 1970's, many innovations were happening in laboratory, computers were changing the way we did things. Previously patient results were manually typed and Xeroxed by secretaries ready to be posted. This progressed to results being written onto key punch cards for data entry staff to enter. Today staff are able to directly to enter results onto the patient computer record and sent paperless by email to the practitioners' own personal computer system or result repository. Some technologists took to the new computer technology and because of their background knowledge in laboratories were able to write relevant and effective software code.

Innovation was happening in other areas of society. You may remember Led Zeppelin's Stairway to Heaven, first heard live in Belfast March 5 1971 with Jimmy Page playing his famous Gibson double necked guitar. This is still one of the biggest selling, single pieces of sheet music, with approximately 15,000 copies sold every year.

Quality assurance accreditation in New Zealand was legislated for in 1972 and the Testing Laboratory Registration Council was formally established the following year, adopting Telarc, New Zealand as its operational name. Telarc became the second such body in the world and was closely modelled on the Australian accreditation body, NATA. NZ medical laboratories became accredited based on the ISO 9002 standard which has been upgraded from time to time. The current 15189 standard is used by laboratories as a reference point to deliver a world class laboratory service to their patients.

The NZIMLS also had to change to keep up with these developments over the years. It introduced a QTA examination to give some direction and control to the number of assistants who had been employed. This was a major undertaking setting up syllabi for all the disciplines took some time and effort. There has been much debate over the years over whether the NZIMLS should continue supporting the QTA examinations and I am pleased to see the examination continuing and indeed now is recognised by the Minister of Health as a registrable qualification.

Medical laboratory scientists' training has changed from the 5 years at the bench apprenticeship training to a 4-year, fulltime university course. Students must now invest significant time and money to complete the course, along with other improvements it does allow a stepping stone onto higher qualifications which a number of graduates have taken.

This Stairway to Science required the NZIMLS to be more involved in the support of its members and following the spin-off of the industrial responsibilities, the Special Interest Groups (SIG) were set up in the 80's to foster continuing education allowing the scientists to keep abridge of new thinking and knowledge. The SIG structure has spread the responsibility down from Council to the scientists and has worked very well with good support from the members. The current CPD programme which evolved from the original Board's MOLS programme is now well supported financially, but will require significant investment in the future to provide a comprehensive array of courses each year so that the members can fulfill their statuary competency obligations.

On the 14th July last year, the Auckland DHBs made a decision which was of major importance to Aucklanders. While this was a productive time for the cartoonist, unfortunately the laboratory science publicity was for all the wrong reasons. This Stairway to Science that had been built up in Auckland was under serious threat. At this time most laboratories throughout the country were undergoing similar upheavals.

This decision had a high impact on the industry as a whole. On that Friday (Bastille Day) the ADHB signed an agreement for community laboratory testing for an interval of 8 years for a sum of $565M with an Auckland Pathology Consortium that had no laboratory, no staff, no equipment, no computer system but was confident this was all to be in place within 12 months. Quite obviously quality issues were not high on their agenda even though Audit NZ with their brand line of "rest assured" had been involved and given the deal its tick of approval. The ADHB had referred on a number of occasions in their RFP to the requirement of a "high quality", "comprehensive quality pathology service", "maintaining or improving service quality", "quality, cost effective and sustainable pathology service". All this had been ignored with the prospect of saving money.

The senior scientists at DML were to be quite frankly insulted by this decision, having devoted their entire youth and adulthood to the task of mastering that peculiar mixture of craft and science that is medical technology they found themselves being treated as though their work was of no particular account, or worse still as wasting resources performing unnecessary laboratory tests. It was not right that a group of individuals who had provided a world class laboratory service for more than 70 years to the Auckland community should be dumped. It was simply not right. The managers wrote a letter to their colleagues stating that they were committed to staying at DML and had no intention of moving to the new lab and see their history be destroyed. It is this passion for the profession and commitment to patients that ultimately won the day against a change that came very close to happening.

There are still challenging times ahead for laboratory services in New Zealand, with health expenditure per capita held below other OECD countries and laboratories are operating under capped budgets. In the Wellington Capital Coast region, the patients of private specialists are no longer eligible for public funded laboratory testing and in the Bay of Plenty the community contract excludes "wellness testing", even though this appears to be in conflict with the medical conditions targeted under the primary healthcare strategy such as diabetes, renal and cardiovascular disease.

There clearly needs to be a NZ overall plan driven by the Ministry so that the laboratory profession can continue in an organized way. At present there is little incentive for investment, both financially and professionally and these major uncertainties are having an affect on the recruitment and retention of our essential laboratory workforce.

I believe the Minister should observe what is happening in Australia where there is significant investment and an overall plan for laboratory services. At present the country is stuck with the confusion. The Minister needs to address this situation urgently.

I believe the NZIMLS needs to continue to work closely with AIMS on a number of these issues, these combined meetings are a splendid opportunity to discuss mutual problems and find solutions. In all areas of NZ society there is an increasing closer relationship with Australia which I fully support. We must maintain our close ties with the AIMS working together in such areas as continuing education to continue this Stairway to Science. I wish you well with your congress, make the effort to build new contacts with other laboratory professionals and do what you can to further our two great Institutes.

Kia horo te marino
Kia papa-pounamu te moana
Kia tere te karo hirohi

May calm be widespread
The sea like greenstone
And may the shimmer of summer
Dance across your pathway

Australian Journal of Medical Science Abstracts

Catchpoole D, deFazio A, Deveraux L, Fleming M, Hof M, Schmidt, Thorne H, Zeps N. The importance of biorepository networks: the Australian Biospecimen Network – Oncology. *Austr J Med Sci* 2007; 28: 16-20.

The value of banking human tissue samples for research is recognised as necessary for progress into understanding human disease and has let to the establishment of biorepositories throughout the research community. While single institution tissue banks have been highly beneficial, it has become clear that information and resource sharing between banks has great potential to increase the number and overall value of tissue specimens available to medical researchers. Recognising this potential for enhancing already valuable tissue resources led to the establishment of the Australian Biospecimen Network (ABN) in late 2001 and the ABN-Oncology consortium in 2004. The ABN seeks to build on existing cooperation and collaboration by establishing an Australia-wide network of tissue biorepositories that collect cancer related tissue using established and accepted guidelines and protocols. The coordinated network of tissue banks has led to a significant enhancement of biospecimens for research that will eventually increase our understanding of human diseases including cancer.

Leong TY, Leong AS. Variables that influence outcomes in immunohistology. *Austr J Med Sci* 2007; 28: 47-59.

Despite entering its fourth decade of existence, immunohistology as a diagnostic tool continues to experience poor reproducibility. This problem is highlighted as the technique is employed as a quantitative assessment of therapeutic and prognostic markers in a number of neoplasms. While qualitative applications continues to increase with the range of sensitive antibodies available, numerous variables that influence the immunoexpression of proteins in formalin fixed exists in the pre-analytical and analytical phases of the test procedure. Pre-analytical variables are currently beyond the control of the laboratory. Tissue fixation is critical but exposure to fixative prior to accessioning in the laboratory cannot be controlled. Antigen retrieval, another pivotal procedure in immunohistology, continues to be employed in an empirical manner with the actual mechanism of action remaining elusive. There is great variation in reagents and duration of tissue processing and the immunostaining procedure is not standardised between laboratories. Variables in interpretation exist and cutoff thresholds for positivity are variable. Failure to recognise false positive and false negative stains leads to errors of qualitative interpretation. Many of the problems related to the technology and interpretation of immunostaining originate from failure to recognise that this procedure is different from other histological stains. Accurate and reproducible quantitative assessment of immunostains remains elusive as important variables that impact on antigen preservation in the paraffin embedded biopsy currently cannot be controlled.

Gardner S, Kewley R, Car G. Insulin resistance. *Austr J Med Sci* 2007; 28: 60-74.

Insulin resistance has many causes, and is difficult to define. The most easily defined role for insulin is the promotion of glucose removal from the blood, therefore much of the discussion on insulin resistance has been focused on glucose. This review attempts to outline the currently accepted mechanisms of insulin resistance, insulin receptors, and insulin signalling. The main focus has been directed at the genetic links via thrifty genes, type 2 diabetes, the metabolic syndrome, the degree of overweight and obesity, lipids, cardiovascular disease and hypertension, polycystic ovary syndrome, and the diagnosis and treatment of insulin resistance.

Gardner S, Kewley R, Car G. A profile of patients suspected of having insulin resistance. *Austr J Med Sci* 2007; 28: 75-82.

The insulin resistance syndrome has been defined as a cluster of hypertension, cardiovascular disease, dyslipidaemia and impaired fasting glucose in individuals with raised fasting plasma insulin levels. The development of insulin resistance may be genetic, or due to modifiable environmental factors such as weight increase and/or lack of physical activity. The aim of this study was to investigate possible relationships between insulin resistance, glucose impairment, obesity and life style factors in a small community in rural Australia. This study highlighted that 89% of the 53 participants, who were referred by their general practitioner for testing for insulin resistance, were overweight or obese. Total cholesterol and triglycerides were raised in 25% of participants, with high density lipoprotein-cholesterol (HDL) levels lower than the suggested guidelines of the National Heart Foundation. Impaired fasting glucose was found in 20% of participants, with 49% of participants having a family history of diabetes. Insulin resistance was demonstrated in 59% of participants with a homeostasis assessment model (HOMA index) of ≥2.2 and 7% of participants were borderline insulin resistant.

Lean SY, Adams MJ. A role for the tissue factor pathway in the pathogenesis of the antiphospholipid syndrome. *Austr J Med Sci* 2007; 28: 99-107.

The antiphospholipid syndrome (aPS) is a systemic autoimmune disorder that is characterised by laboratory markers of antiphospholipid antibodies (aPL) as well as clinical manifestations such as arterial and venous thrombosis, recurrent pregnancy loss and thrombocytopaenia. Although it is unclear precisely what mechanism(s) lead to thrombosis in aPS, it is probable that the contribution of aPL such as anti-ß-2-glycoprotein-1 (anti- ß2GP1) is significant. Indeed, there is increasing evidence that aPL may interfere with the tissue factor (TF) pathway of blood coagulation and its natural regulator tissue factor pathway inhibitor (TEPI), thus contributing toward the development of thrombosis in aPS. This paper will therefore review 1) the TF pathway of blood coagulation and TFPI, 2) clinical and laboratory aspects of aPL and aPS, and 3) interactions of aPL with the TF pathway, to highlight the potential significance of these in the pathogenesis of aPS.

Cooper S, Zarkos K, Jurinkulravanish T, Dolotin M, Brown R. Diagnosis of hereditary spherocytosis by flow cytometric detection of eosin-5'-maleimide binding to band 2. *Austr J Med Sci* 2007; 28: 109-11.

A screening test for hereditary spherocytosis (HS) using the dye eosin-5'-maleimide (EMA) has been evaluated in our laboratory, which previously used both the hypertonic cryohemolysis and acidified glycerol lysis assays. The test is rapid, easy to perform and is the test of choice for laboratories that routinely perform flow cytometric assays.

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The Pacific Way

A three week clinical biochemistry course was held at the PPTC from 28 May -15 June 2007. There were six participants: Sero Kalkie from Vila Central Hospital in Vanuatu, Paul Makikon from the Northern District in Vanuatu, Gretna Tauma from Tungaru Central Hospital in Kiribati, Trusty Boisek from the Palau National Hospital, Palau, Makerita Sooalo from Medcen Hospital in Apia, Samoa and Ioichi Aut from Chuuk State Hospital, Federated States of Micronesia.

This was only the second biochemistry course to be held at the PPTC the first being held in 1986. The major emphasis of this course was on non-communicable diseases which are becoming a major health issue in the Pacific Island countries as they are in the rest of the world. Lectures were given to the participants by laboratory staff of Wellington Hospital on topics such as heart, thyroid, renal diseases and diabetes. Representatives from several companies who have biochemistry analysers in Pacific Island laboratories gave demonstrations of their equipment and also discussed maintenance and trouble shooting issues. Lectures were also given on specimen collection, send-away specimens, QC, EQA, standard operating procedures, stock control, laboratory information systems, and staff relationships. The students had tours of the Wellington Hospital laboratory, NZ Blood Service Wellington Centre, and Wellington Hospital Emergency Department. At the end of the course Dr Michael Crooke, Chemical Pathologist, presented certificates to the course participants and congratulated them on their attendance and participation in the many and varied lectures.

Participants of the clinical biochemistry visiting the Wellington Hospital laboratory

Blood bank technology course

A very successful blood bank technology course was held during August. We had three participants: Mine Kojet from Majuro Hospital in the Marshall Islands, Osborne Somo from Lata Hospital in the Solomon Islands, and Michael Taloifaga from Kiluufi Hospital, Solomon Islands . The PPTC is very grateful to the Wellington Area Centre of the NZ Blood Service for again running the training programme and especially John Dagger for all his hard work. Mrs Jeanette Watson, Team Leader Blood Bank, presented certificates to the participants at the end of the course and encouraged them to continue working hard to keep Blood Transfusion safe in the Pacific.

Participants of the clinical biochemistry course and friends of the PPTC.

Participants of the 2007 blood bank technology course.

Minister of Foreign Affairs Pacific Mission:

Rob Siebers, a PPTC Management Committee Member, recently was chosen to represent the PPTC on the Minister of Foreign Affairs' (the Rt. Hon Winston Peters) Pacific Mission.

The delegation was made up of the Minister of Foreign Affairs, other Ministers and Members of Parliament, Ministry officials, academics, non-Government Organisations, academics, and journalists. Over a period of six days, the delegation visited the Solomon Islands (1 day), the Marshall Islands (1 1/2 days), and Samoa 3 days).

The delegates had a very hectic schedule, with many meetings with government and ministry officials in those three Pacific countries, as well as visits to New Zealand Aid projects. Rob did, however, find some time to visit the laboratory staff at the main hospital in Samoa.

Overall, it was a good opportunity to showcase the PPTC in those countries as well as to the other delegates. In most instances, the PPTC's name and reputation was already well known.

Journal article questionnaire

for the Haematology Special Interest Group section of the NZIMLS journal, Issue November 2007

Morie A. Gertz. "Management of cold haemolytic syndrome"

British Journal of Haematology, June 2007, Vol 138, p 422-429

Questions:

1. The antibody of paroxysmal cold haemoglobinuria (PCH) was first described by who and when?
2. What class and specificity has the PCH antibody?
3. PCH affects mostly what age group?
4. The haemolysis in PCH is extravascular, True or False?
5. Splenectomy has no role in treatment for PCH, True or False?
6. What is the name of the specific test for PCH?
7. The test for PCH depends on what characteristic of the antibody?
8. What modification of the PCH test enhances detection of low titre antibodies?
9. The test for PCH may be initially negative, True or False?
10. What is the class of antibody present in cold agglutinin disease?
11. What is the main specificity of the antibody in cold agglutinin disease?
12. The haemolysis in cold agglutinin disease is intravascular, True or False?
13. Post infectious cold agglutinins are most notably associated with which two conditions?
14. Haemolytic anaemia associated with monoclonal cold agglutinins is less serious than that of polyclonal cold agglutinins, True or False?
15. Why is cold agglutinin haemolysis more resistant to therapy than warm antibody mediated haemolysis?
16. What are typical laboratory features of cold agglutinin disease?
17. What therapeutic drug has shown a favourable response in cold agglutinin disease?

Questions prepared by Jacquie Case, Haematology Dept., Middlemore Hospital. For a copy of the journal article, Ph 09 276 0044, xtn 8515 or e-mail jcase@middlemore.co.nz

Answers on page 94

President's report

This financial year commenced with a significant initiative for Council, a review of the structure of the Executive Office. Recent years have seen a significant increase in workload for Fran and the team in the NZIMLS Executive Office. This has been attributable to a marked increase in Institute membership, which has grown over the last three years from four hundred and fifty members to the current membership of approximately two thousand. A significant contributory factor has been the uptake of the CPD programme by medical laboratory scientists for whom participation, and satisfactory performance, in a recertification programme is mandatory for the issuance of an Annual Practicing Certificate. Additional factors impacting on the workload of the Executive Office have included an increase in the number of Special Interest Group meetings, including the addition of the North Island Seminar, the introduction of the QPT and QSST examinations, greater reliance on electronic information systems, in particular the Institute's website, and lack of standardisation of accounting systems between the Executive Office and the Institute's accountants. During 2006 it became apparent to Council that additional resource was required and to this end the review of the structure of the Executive Office was undertaken. Council considered a number of options for the restructured Executive Office that would provide members with the best possible level of service, while having due regard to the most prudent use of the financial resources of the Institute. In a significant departure from the Institute's longstanding contractual arrangements for the provision of Executive Officer and secretarial functions, Council recommended that the Institute move to directly employ the Executive Officer plus an additional position of Database Administrator/Accounting Technician, these both to be fulltime positions. Additional resource for specialist functions to be provided on a sub-contractor basis. The Institute now has sub-contracts for the positions of: CPD Co-Coordinator (20 hours per week), Webmaster (10 hours per week), IT Support (10 hours per week), Assistant to Executive Officer and Database Administrator (10 hours per week) and Journal Advertising Manager. The provision of office space and equipment for the Executive Office is by way of a contract with eevents. Council is delighted with the functioning of the restructured Executive Office, and members can be assured that they are being well served. While the positions of the elected Councilors are transitory, for NZIMLS members Fran, as Executive Officer, is identified as the face of the Institute. It is to the great benefit of the organisation that, with the restructuring, Fran has chosen to continue in the role of Executive Officer.

An important issue identified during the restructuring process was the need to formally document the key functions and processes undertaken by Council and the Executive Office. Two members of Council, working with the team in the Executive Office, have made an excellent start on the preparation of a quality manual system of documentation. When completed the comprehensive documentation will minimise risk to the Institute as well as ensure that robust systems are in place for auditing purposes.

During the year the NZIMLS has had contact with the Australian Institute of Medical Science (AIMS), including a meeting between the President and Executive Officer and the AIMS Council at the AIMS/AACB Annual Scientific meeting. One issue that we have raised with AIMS is a request for New Zealand Medical Laboratory Scientists with qualifications predating the BMLSc, ie COP or Diploma in Medical Laboratory Technology, to be recognised for membership of AIMS at the Professional level. The AIMS National Council has notified us that they will be setting up a working party to look at this and the broader issue of grandfathering of qualifications and requirements for AIMS membership.

In recent years the NZIMLS Council and the NZ Medical Laboratory Science Board (MLSB) have agreed to schedule a yearly joint meeting, with both bodies finding this to be a particularly useful meeting. This year's meeting was held in conjunction with the May Council meeting and gave the Council the opportunity to raise a number of issues with the Board, including the status of technicians working in the specimen services section of laboratories. Council is firmly of the opinion that there is a significant potential risk of harm to the public for errors occurring in the specimen services section of the laboratory, and that specimen services technicians holding a QSST should be registered. The Board has recommended that the Institute submit a proposal to this effect, for consideration, and a Council working party has been established to action this. The moderation of MLS disciplines for the BMLSc degrees was discussed, with Council noting the difficulties in finding appropriate scientists to perform this important function. To improve this, where possible, moderation schedules will be developed that allow for the moderation of a single subject across the three universities. In recognition of the commitment required by moderators, Council has approved an additional payment to moderators, matching the fee paid by the universities.

The rationalisation and reconfiguration of laboratories throughout the country has seen a drop in the number of participants enrolled in the CPD programme, with 1,451 enrolled this year compared to 1,690 in 2006. This year will see the completion of the initial three year cycle of the programme. With this in mind Council has undertaken a significant review of the categories for CPD activities as well as the associated allocation of points. The updated recertification programme has been approved by the MLSB and published in the My CPD section of the website. An updated CPD booklet will be produced for 2008. It is very heartening to see the universal acceptance of the programme by practitioners. Those participating are now well familiarised with the both requirements of the programme and the annual audit process. Submissions for the latter have been greatly simplified this year with the provision of a downloadable audit sheet for confirmation of competency based on the practitioner's record of laboratory training documents. Council is committed to providing continuing education opportunities that will assist practitioners in meeting their CPD requirements. To this end, the MLS Classroom has been developed as an on-line educational tool. The Classroom utilises multi-choice questions provided through the NZIMLS website. At least 100 questions are required for each discipline. The project has been piloted in Haematology and the questions for this discipline are almost complete. Questions are required for other disciplines, and in order that the project might advance more quickly I would encourage members to consider submitting suitable questions. Details about the classroom and examples of the type of questions that are being sought can be found under the Classroom section of the website.

As the financial year closes, I would like to acknowledge the commitment of my fellow Council members, as well as the dedication of those Institute members who have assiduously given of their own time for the betterment and progression of the profession.

Robin Allen

President, NZIMLS

New Zealand Institute of
Medical Laboratory Science

Minutes of the 63rd Annual General Meeting held at SkyCity Auckland Convention Centre on Thursday 23RD August 2007 commencing at 7.30am

Chairman

The President (R Allen) presided over the attendance of approximately 90 members.

Apologies

Moved J Wypych, seconded P Austin
That apologies be accepted from David Bunker, Martin Hampson, Mike Lynch, Ron McKenzie and John Elliot.
Carried

Proxies

Moved R Hewett, seconded C Pickett
That the list of three proxies as read by the Secretary be received.
Carried

Minutes of the previous AGM
Motion:
Moved A Buchanan, seconded J Wypych
That the Minutes of the 62nd Annual General Meeting held on 24th August 2006 be taken as read.
Carried

Motion:
Moved R Hewett, seconded A Buchanan
That the Minutes of the 62nd Annual General Meeting held on 24th August 2006 be confirmed as a true and correct record.
Carried

Business arising
Nil.

Remits
Motion:
Moved R Hewett, seconded C Kendrick
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.
Carried

Motion:
Moved R Hewett, seconded C Kendrick
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.
Carried

Presidents report
Motion:
Moved R Allen, Seconded R Siebers
That the President's Report be received.
Carried

Annual report
Motion:
Moved M Legge, seconded W Dellow
That the Annual Report be received and adopted.
Carried

Financial report
Motion:
Moved R Hewett, seconded C Kendrick
That the Financial Report be received and adopted
Carried

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

President	Robin Allen
Vice President	Kevin Taylor
Secretary/Treasurer	Ross Hewett
Region 2 Representative	Chris Pickett
Region 4 Representative	Sandy Woods
Region 5 Representative	Anne Buchanan

The results of the elections for:

Region 1 Representative	Joe McDermott	21
	Paul Austin	23
	Margaret Matson	25
Region 3 Representative	Gloria Crossley	18
	J Wypych	37

Motion:
Moved R Hewett, seconded T Rollinson
That the Election of Officers be approved.
Carried

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

Qualified Medical Laboratory Technician awards

- Clinical Biochemistry - Cameron Walker, Gribbles Alpha, Hamilton
- Haematology – Beth Caudwell, Diagnostic Medlab, Auckland
- Histology – Carolyn Hunter, Diagnostic Medlab, Auckland
- Immunology – Rebecca Dew, Canterbury Health Laboratories, Christchurch
- Microbiology – Suzi Betteridge – Aotea Pathology, Wellington

- Transfusion Science – Dianne Gillard, Medlab Bay of Plenty, Tauranga
- Transfusion Science, Blood Products – David Currie, New Zealand Blood Service,Wellington
- Mortuary Hygiene & Technique – Bo Tindall, Canterbury Health Laboratories, Christchurch

Qualified Phlebotomy Technician
QPT – Tracey Mayall, Medlab Bay of Plenty, Tauranga

Qualified Specimen Services Technician
QSST – Rena Upadhyay, LabPlus, Auckland

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

Carried

Auditor
Moved R Hewett, seconded K Taylor
That Hilson, Fagerlund and Keyse be appointed as the Institute's auditors for the 2007/2008 financial year.

Carried

General business
John Aitken outlined to the meeting, events that had occurred since the previous AGM with regard to laboratory restructuring around the country. He noted that the District Health Boards (DHBs) will redo their strategy and will not make the same mistakes again and he is therefore, now concerned that we are seen to be taking sides with the DHBs and that it will be difficult to comment publicly.

J Aitken noted the following:

- Appropriate ways of increasing profile of medical laboratory scientists and in particular the two training.
- Issues of not enough students coming through and not enough experienced medical laboratory scientists to train the students.
- Burn out is an issue.
- There is pressure on medical laboratory scientists not to speak out as the Government only wants to hear good news. Suggested canvassing the opposition and minor parties.
- Often requires an acute event to get media attention. Suggested reading the newspapers and see what can be commented on via a press release with something amusing to catch attention, eg chemicals in China goods.

A Bunker considered that a step by step plan is required.

It was noted that Council have put together two promotional pamphlets – one for Schools (careers) and the 'Who are We' flyer for walls of Phlebotomy rooms etc.

Garry Milicich, Wellington is pleased to hear an aggressive voice coming from our profession and feels we need to be very aggressive with politicians. Mike Legge, Dunedin commented that if people want to approach opposition or a Member of Parliament these people need to know beforehand and be briefed very well as probably may not have a sufficient level of knowledge of the subject. J Aitken suggested that only a one page briefing paper is required.

Gloria Crossley feels that the Institute has to do more and needs to be feed from the top and feed onto members. Members need to know what you want us to take to our local MPs. Considered that there is not enough togetherness and requires a lot from information from Council. She is particularly concerned about the appalling Innovative Project questionnaire noting that it does not support members as scientists, its supports pathologists. Considered that it is a DHBNZ problem as they seem to believe that there are enough medical laboratory scientists in the workforce.

C Kendrick spoke in favour of the DHBNZ initiative as we do need a structure in our profession and this is an ideal as professional. The survey is important to get feedback from the profession and very innovative.

A Bunker suggested that we do not to underestimate the value of the public as it was not the Board that got the registration of the QPT and QSST – it was the public.

D Fairfoot agreed with the comments being coordinated and together, and suggested that a PR company become involved.

Motion:
Moved D Fairfoot, seconded A Bunker
That the Institute consider whether the finances are available to take on a PR company to promote the profession.

Venue for the 2008 Annual General Meeting and the Annual Scientific Meeting
St David's Lecture Theatre, University of Otago, Dunedin

Venue for the 2009 Annual General Meeting and Annual Scientific Meeting

R Siebers offered Wellington as the venue for the 2009 Annual General and Scientific Meeting.

There being no further business, the Chairman closed the meeting at 8.20am.

Journal-based questionnaire

Journal-based questionnaire for the November 2007 issue

Below are 10 questions based on articles in this issue of the Journal. Read the articles carefully, most questions require more than one answer.

Answers are to be submitted through the NZIMLS web site (www.nzimls.org.nz). Make sure you supply your correct email address. The site will remain open until Friday 18 January 2008. You must get at least 8 questions fully right to obtain 5 CPD points.

Journal-based questions:

1 Name the basic steps for the extraction of DNA/RNA from commercial columns.

2 What is required to synthesise new dsDNA and what does it facilitate.

3 What steps does the reverse transcription PCR process consist of.

4 Which instruments are combined into one for real time PCR.

5 Name an example of strand displacement amplification.

6 How does the ligase chain reaction differ from PCR.

7 Which resistant strains belonging to the *Eschericia* genus were isolated from chickens in Millar's study.

8 What are the possible transmission routes of antibiotic resistant bacteria.

9 What is the unique characteristic of the sideroblastic anaemias and what is it the result of.

10 What are the effects of alcohol on haematopoiesis.

Questions and answers for the August 2007 journal-based questionnaire

1. The screening programme for prostate cancer for South Indian males emphazises the importance of which three approaches. **Digital rectal examination, ultra sound and PSA measurement.**

2. How was the serum PSA measured in the first case study. **With a one step double monoclonal antibody enzyme immuno- (ELISA) using the Enzymun-Test Kit on the Boehringer Mannheim ES300 immunoassay analyser.**

3. What is the protocol in MedLab Hamilton if an HDL cholesterol of <0.5 mmol/L is found. **Look at the reaction monitor and history of the patient. If the patient is not a known myeloma case, and the reaction monitor is abnormal, then a total protein is added and the specimen sent to immunology for protein electrophoresis.**

4. PSA is considered as an ideal tumor marker for what. **For monitoring prostate cancer and assessing response to treatment**.

5. What is MedLab Hamilton's criterion for protein electrophoresis. **A total protein of > 80 g/L and/or a globulin of > 40 g/L, without a known patient history.**

6. In the diagnostics footprints article what were the bone marrow findings and what diagnosis was made. **A marked number of myeloma cells comprising 85% of total white cells. Many of these plasma cells were binucleated with some multinucleated forming plasmacytomas. A diagnosis of advanced multiple myeloma was made.**

7. What is Warrick Nelson and Ben Harris' view on the likely source and vectors for Campylobacter in New Zealand. **Animal (including cows, sheep, pets and humans) and bird excreta. Vectors: water, flies and sparrows.**

8. What is the main Campylobacter species found in campylobacteriosis cases and at what percentage. What other organism has been implicated. **Campylobacter jejuni, about 80%. C. coli, at least 10%**.

9. By how much did the incidence of Campylobacter infection in the Belgian population drop by from the expected rate when poultry was removed from the market in 1999. **40%**.

10. The *Campylobacter* organism is part of the normal gut flora only in chickens raised in sheds. False or true. **False**.

Massey University - NZIMLS prize for 2006

The winner of the NZIMLS top academic award in year three of the Massey BMLSc in 2006 was Joshua Stevens. The award was to the value of $2000.00 and is offered annually by the NZIMLS to support students in the clinical placement year of the degree. Joshua is currently completing his clinical training at Princess Alexandra Hospital in Brisbane where he is studying Microbiology and Haematology.

Joshua was born in Napier and raised with brother Sam by Bob and Sue Stevens. Early days growing up were spent on an orchard before the family moved to live in the suburb of Ahuriri. Joshua commenced his studies in Veterinary Science at Massey University in 2004 but shortly after switched to the BMLSc programme. Joshua grew up with a keen interest in cricket, something he says he would still be playing if it weren't for the time constraints of study and the BMLSc degree.

Joshua would like to thank the NZIMLS for this award and the many academic and technical staff at the University for their mentoring and support throughout the programme. In addition he acknowledges the support of his family and fellow BMLSc students at Massey. The many unforgettable and enjoyable social moments of the past years will be fondly remembered.

The NZIMLS congratulates Joshua on his award and wishes him all the best for the future.

Answers to the HSIG journal questionnaire:

1. Donath and Landsteiner in 1904.
2. IgG antibody, Anti P specificity.
3. Young children.
4. False, it is intravascular.
5. True
6. Donath Landsteiner test.
7. The Donath Landsteiner test depends on the fact that the anti P antibody binds to red cells and fixes complement at 3°C. Subsequent warming to 37°C allows C4 –C9 activation leading to red cell lysis.
8. Addition of papain treated group O red cells.
9. True.
10. IgM.
11. Anti I.
12. False, it is extravascular.
13. Mycoplasma pneumonia and infectious mononucleosis.
14. False.
15. Because of the density of complement on the red cell surface being relatively high.
16. Extremely elevated MCV (as high as 140fl), hyperbilirubinaemia, elevated LDH levels and a positive direct coombs test.
17. Rituximab.

Advertisers in this issue

New Zealand Institute of
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NZIMLS Fellowship

Members of the NZIMLS may not be aware of a recent change to the Fellowship regulations. It is now possible for candidates to be exempted from the Part 1 examination and to obtain Fellowship by Part II if they are holders of an appropriate postgraduate qualification.

Rule 3.17 of the Fellowship Regulations states:

Part 1 by exemption. Candidates applying for Fellowship by examination may be exempted the Part 1 examination if they are holders of an approved postgraduate qualification in Medical Laboratory Science. The course of study must meet the minimum requirement of the equivalent of one year's full time study.

Post graduate qualifications recognised by the Institute, for purpose of exemption to sit the Part 1 examination are:

- ***Fellowship of The Australian Institute of Medical Science (AIMS)***
- ***Fellowship of the Institute of Biomedical Science (IBMS)***
- ***Fellowship of The Australian Association of Clinical Biochemists (AACB)***
- ***A postgraduate qualification in Medical Laboratory Science, or an appropriate postgraduate qualification approved by the Fellowship Committee***

Approval of other qualifications will be at the discretion of the Fellowship Committee

Check out the NZIMLS web site for the full regulations for Fellowship.

Chris Pickett, Rob Siebers, Ann Thornton. Fellowship Committee.

www.nzimls.org.nz

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1. Data on file.
2. Walboomers J, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol.* 1999;189:12-19.