

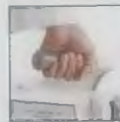
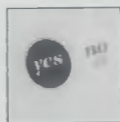
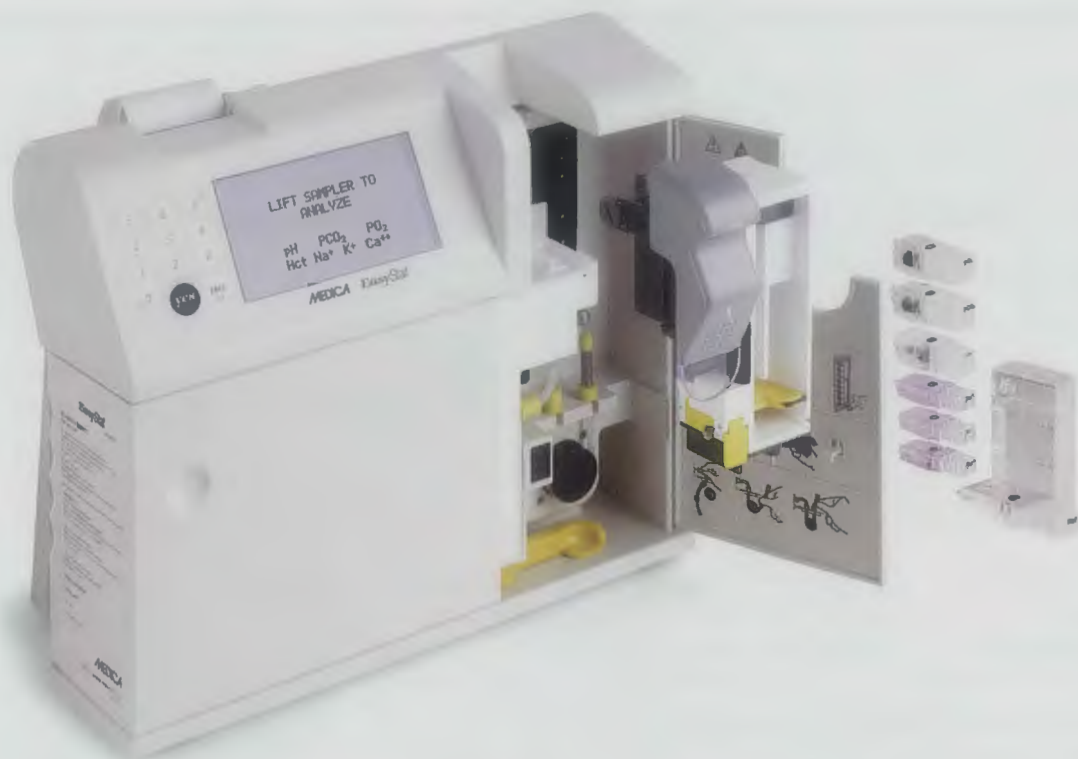
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# 2

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# Application of a PCR method in a pilot study investigating *Chlamydia pneumoniae* in a CAP population. From the Waikato - Canterbury CAP study 1999-2000

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## Abstract

A prospective cohort study was conducted at both Christchurch and Waikato Hospitals in 1998-1999 on patients admitted with Community Acquired Pneumonia (CAP). Disease incidence was ascertained in the specimens of these patients using traditional and a new molecular detection method. The incidence of *Chlamydia pneumoniae* was obtained by using PCR for rapid diagnosis and compared to the MIF serology test often referred to as the "gold standard".

Total genomic DNA was extracted from WBC and throat swabs. The extracted DNA was amplified by PCR using specific primers against the 16s rRNA gene of *Chlamydia pneumoniae*. The assay had a confirmatory step, using nested PCR. Chemical parameters were titrated and thermocycling and temperature conditions were optimised for the detection *Chlamydia pneumoniae*. This resulted in an analytical assay sensitivity of 1 CFU/ml. This sensitivity was comparable to other published PCR assays. PCR provided results within one day compared to several weeks for serological assay.

The methodology developed was able to provide accurate and specific rapid results, which, have the potential to assist in the delivery of health services. The results of this PCR assay for the detection of *Chlamydia pneumoniae* were then compared to the serology MIF test, and analysed for disease incidence.

Earlier New Zealand CAP studies, using serological diagnosis or culture only had found little incidence of *Chlamydia pneumoniae*. Results from this current pilot CAP study found that there were no cases of *Chlamydia pneumoniae* in the specimens assayed, despite the more sensitive, specific PCR technology. The serological MIF also found no positive results. The full bank of specimens was not tested due to the lack of positive results and the relevance of testing.

In summary, previous CAP studies had an incidence of *Chlamydia pneumoniae* of up to 3%, whereas the current pilot CAP study 1999-2000 had an incidence of <1% by serology and PCR.

## Introduction

### Overview.

Pneumonia is responsible for a significant number of hospital admissions, and it is therefore of clinical importance to diagnose the specific cause. Public health initiatives also require definitive diagnosis to ensure the appropriate treatment is given. *Chlamydia pneumoniae* causes up to 20% of community acquired pneumonias (1,2). Definitive diagnosis has been by isolation of the organism in cell culture followed by immunohistochemical identification with a *Chlamydia pneumoniae* specific monoclonal antibody. However culture techniques lack sensitivity (3-5) and take several days. Alternatively, retrospective diagnosis can be achieved by serology using the microimmunofluorescent test (MIF). However the MIF test relies on an antibody response to reach a threshold level and therefore is not an early-detection method. Detection of *Chlamydia pneumoniae* by molecular assay using the polymerase chain reaction (PCR) has the potential to offer increased sensitivity and specificity compared to culture, with a reliable result within 24hours.

## Epidemiology of *Chlamydia pneumoniae* pneumonia within the New Zealand setting

*Chlamydia pneumoniae* has been recognised as a significant cause of community-acquired pneumonia (CAP) since the 1970's. In a 1988 CAP study of hospitalised patients reported in New Zealand (6) the incidence of this pathogen was compared to the incidence of *Streptococcus pneumoniae* (common pneumonia). The incidence was *Mycoplasma pneumoniae* 18%, *Chlamydia* spp. 2%, *Legionella* spp. 4%, viruses 11%, and *Streptococcus pneumoniae* 33%. Similarly a 1992-1993 Christchurch based study showed the following prevalence *Streptococcus pneumoniae* 39%, *Mycoplasma pneumoniae* 16%, *Chlamydia pneumoniae* 3%, *Legionella* spp. 11% (7,8) Both these studies diagnosed the incidence of *Chlamydia pneumoniae* by the MIF serology test or culture alone and were never confirmed by an alternative technique.

## Objectives of this study

The purpose of this study is to provide appropriate laboratory diagnostic techniques to obtain a statistical profile of infection by *Chlamydia pneumoniae* in a pilot CAP study population. The results of two methods will give an insight into the incidence of *Chlamydia pneumoniae* within a New Zealand pneumonia population. This incidence was compared to previous New Zealand studies and to the incidence of disease overseas.

The two methods of diagnosis that were considered in this study were **1.** molecular detection of Chlamydial DNA using PCR and **2.** the MIF test, serological detection of Chlamydial antibody, which is considered the "gold standard."

## *Chlamydia pneumoniae* the bacteria

*Chlamydia pneumoniae* is an obligate intracellular parasite classified as a bacterium because of the composition of the cell wall (9). *Chlamydia pneumoniae* has more recently been renamed *Chlamydophila pneumoniae* (10). As this terminology has not been widely published to date I will continue to use the more familiar term. The genus *Chlamydia* is represented by three separate species:- *Chlamydia pneumoniae*, associated with respiratory disease and pneumonia; *Chlamydia trachomatis* associated with trachoma, infantile pneumonia and as a sexually transmitted disease; and *Chlamydia psittaci*, associated with acute zoonotic disease involving birds.

The life cycle has two distinct phases: a non-replicating extra cellular infectious stage and an obligate intracellular non-infectious replicating stage. The infectious elementary body (EB), attaches to susceptible host cells and the bacteria enter the cell by phagocytosis. Following cell entry, the elementary body reorganises into reticulate particles (forming inclusion bodies) and binary fusion begins using the host cell's ATP. After 18 to 24 hours the reticulate particles condense to form elementary bodies that are released by the rupture of the host cell to begin another infectious cycle (11).

The chlamydial elementary body possesses genus (group) specific, species specific and serotype specific antigens. The group antigens are most closely associated with the lipopolysaccharide (LPS) of the outer

membrane. This extractable LPS is commonly used to produce group reactive antigens for serological assays. The major outer membrane protein (MOMP) contains species and serotype specific antigens. This protein constitutes approximately 60% of the organism's outer membrane. Several other structural proteins are associated with the chlamydial outer membrane. However the LPS and MOMP dominate the induction of the human immune response (12).

### ***Chlamydia pneumoniae* disease**

*Chlamydia pneumoniae* has been reported to cause community-acquired upper and lower respiratory tract infections, where the organism can be detected in respiratory sites (1) and in WBC (13). Transmission of *Chlamydia pneumoniae* is via respiratory secretions, or asymptomatic carriers (14) and humans are the natural host (9). The incubation period is several weeks and infection occurs mainly in school-aged children and the older population. The nature of the immune response after primary infection is characterised by an initial IgM response within 2-4 weeks. The IgG response may be delayed up to 6 weeks and IgG antibodies can persist for years, so a high stable IgG titre can be indicative an infection months before (15). The secondary *Chlamydia pneumoniae* infection is characterised by an absence of IgM and a prompt IgG and IgA response (15). Persistent elevated IgA levels is considered an indicator of chronic infection.

Most *Chlamydia pneumoniae* infections in adults are re-infections (16). Occurrence is all year round, and infectivity is low. Epidemics are thought to occur every six to seven years (17). Asymptomatic carriage of *Chlamydia pneumoniae* has been detected in pharyngeal swabs of patients with no clinical symptoms. This suggests that either a chronic infection existed (18) or equally possible that the PCR is too sensitive and may be detecting subclinical bacterial load. Some studies have found persistence of the bacteria for some months, despite appropriate antibiotic treatment (9, 19).

*Chlamydia pneumoniae* has also been reported to cause recurrent or secondary lower respiratory tract infections, and to be associated with a variety of disease states including chronic inflammatory diseases such as asthma (20), chronic obstructive pulmonary disease (5), lymphomas (21) and atherosclerotic plaques (22-24,14), although some authors have not been able to confirm the presence of the bacterium in plaques (25,26). *Chlamydia pneumoniae* has been detected in the peripheral blood mononuclear cells of patients with atherosclerosis and coronary artery disease (13), in the CSF of patients with multiple sclerosis (27), in middle-ear aspirates of children with acute otitis media (28), and in reactive arthritis (29).

## **Objectives and design of study**

A prospective cohort study was conducted at both Christchurch and Waikato Hospitals on patients admitted with community acquired pneumonia (CAP). Patients admitted to both these hospitals over that period were entered into the study if they fulfilled the following criteria: **1.** clinical symptoms indicative of pneumonia, **2.** radiological changes indicative of pneumonia. This diagnosis at times may have been confirmed by the microbiological isolation/detection of a pathogen known to cause pneumonia.

A control group of patients also had specimens collected during the same period. These patients were age and sex matched to the test group. Correspondingly this control group did not have symptoms indicative of pneumonia, this was confirmed by chest X-Ray.

There were 474 patients enrolled based on a clinical assessment of pneumonia, 304 of these were from Christchurch and 170 from Waikato. 180 control patients were enrolled in Christchurch. The study was conducted over a period of 18 months from 1998 to 1999.

### **Specimens collected**

Specimens collected from both test and control groups included: throat swabs, sputum, WBC, serum, plasma and urine. These specimens were collected on Day 1 of admission (acute specimens) or as near to admission as possible, before the instigation of antimicrobial therapy, and also 6 weeks after initial admission (convalescent specimens).

### **Specimens tested**

To gain an initial indication of disease incidence the research group responsible for instigating this pilot study proposed testing a limited number of test and control patient specimens by PCR. This reasoning was motivated by the relevance of testing if there were no positive results, rather than a funding issue.

Of the Canterbury cohort a total of 211 pneumonia patients and 45 control patients had PCR performed, acute specimens assayed included WBC and a throat swab collected in PBS. All patient and control groups had chlamydial antibodies assayed using the MIF test.

Testing of acute and corresponding convalescent serum collected six weeks after admission were assayed in parallel to ascertain if there was a diagnostic rise in IgG antibody. A diagnostic rise in IgG was considered where there was no detectable antibody in the acute sample and a fourfold rise in antibody titre was present in the convalescent sample. This was interpreted as an indicator of acute infection. All serology test results were interpreted by the clinical microbiologist.

### **Laboratory diagnosis of *Chlamydia pneumoniae***

Diagnostic methods included culture, serology procedures, and nucleic acid detection using PCR. Replication of the bacterium is cell-associated, this necessitates in-vitro cell culture using HL cells (30) or HEp-2 cells (31). The life cycle takes 33-hour and incubation of the infected cell line takes approximately 40 hours. Clinical specimens are required to be processed rapidly as the organism quickly becomes non-viable at room temperature (i.e. 1% viable after 24hr at room temperature) (19). The isolation of *Chlamydia pneumoniae* remains difficult (32,33) and its sensitivity has not been evaluated (5), thus it is not available in most routine diagnostic laboratories and was not used in this study.

Serological diagnosis included enzyme linked immunosorbent assay (ELISA) which detects IgG, IgM and IgA antibodies using a recombinant DNA lipopolysaccharide (LPS) antigen (5), and complement fixation tests (CFT) also using an enriched LPS antigen (12). This broadly cross-reactive antigen is only able to detect group antibody response. CFTs are technically difficult to perform and are inherently insensitive. Thus these two serological tests are not adequately definitive to distinguish between the chlamydial species (32).

The MIF test is recognised as the "gold standard" (principle diagnostic serological method used) for detecting chlamydial antibodies, however it has been more recently proposed that this test should be confirmed by alternative serology or molecular testing thus creating an "expanded gold standard" (5). The antibody response can often take at least three to four weeks to reach a significant level (9). This method uses the three different chlamydial species as antigens, and is the only serological test that is in any way able to differentiate between the chlamydial species. However this method is known for cross-reactivity between the species, and with other unrelated species (34). The MIF test only detects IgG antibodies.

Some studies have indicated that serology has little clinical use. The MIF test, which is the most common serological assay used, suffers from problems of cross-reactivity. This was confirmed when positive

MIF specimens failed to be positive by PCR (23).

The MIF test utilises purified elementary bodies as the substrate. The purification step removes the genus-reactive LPS antigen. The purified elementary bodies can then be used to detect species and serovar specific chlamydial antibody reactions. The three individual strains of purified elementary bodies are then spotted separately onto a multi-well slide so that a specific immune response to chlamydial infection can be detected. The test principle involves a two stage "sandwich" procedure, using a fluorescein-labelled antibody to IgG (35). Semi-quantitative endpoint titres can be obtained using serial dilutions of serum. This step assists with differentiating a diagnostic rise in chlamydial titre.

The advent of molecular biology has enabled the laboratory to offer a new area for development of rapid diagnostic techniques. Nucleic acid detection of *Chlamydia pneumoniae* by PCR can be performed using primers targeted against various regions of the bacteria, these include:

\* different areas of the MOMP gene (36,37) where the use of target-specific primers enables this technique to differentiate between the Chlamydial species.

\* the highly conserved region of 16s rRNA gene (1,38) where the first authors used an additional nested step with species-specific primers. The target sequence occurs in the 16s rRNA gene in a single copy.

All known human isolates of *Chlamydia pneumoniae* differ by only one position in the 16s rRNA gene, indicating genetic homogeneity among these strains, and thus ensuring that all significant strains will be detected by these primers (39).

The first step PCR (PCR1) using the external primers Cpn 1 and Cpn 4 amplified a 1392 bp product. Confirmation of the target sequence was performed by a second nested step (PCRn), the inner set of primers Cpn 2 and Cpn 3 amplified a 859 bp product, from within the initial 1392 bp product. This second step also increases the sensitivity and specificity of the assay.

## Methods and materials

### Bacterial controls for PCR

*Chlamydia pneumoniae* strains CM 1 and CWL-011 were donated by Mike Brokenshire from Microbiology Department, Auckland Hospital, and maintained on HEp-2 cells with RPMI media containing 10% Foetal Calf serum (FCS) (9).

### Specimens processed.

PCR for *Chlamydia pneumoniae* utilised WBC and throat swabs in phosphate buffered saline (PBS) i.e. without Chlamydia transport media, these sites being where the organism had been detected previously (1, 13). Specimens were collected in the acute phase of infection, and before the instigation of any antimicrobial therapy (which may inhibit the detection of bacterial DNA).

Serology used acute and convalescent paired sera.

### Sample preparation before extraction of DNA.

Throat swabs received in PBS were centrifuged for 10min at 7,500g. The resulting pellet was used for the first step of the DNA extraction.

White blood cells from EDTA blood had the red blood cells lysed first using a RBC lysis buffer (40). After incubation at room temperature for 15min, the sample was centrifuged for 10min at 7,500g. The resulting pellet was washed twice with PBS and the final pellet re-suspended in PBS for DNA extraction.

### Purification of genomic DNA

The QIAamp Mini extraction kits are designed for the rapid purification of total DNA (41,42).

Purification of DNA was as per the included instruction sheet. In summary throat swabs and WBC's were added to lysis buffer and Proteinase K and incubated. The lysed specimens were then applied to the spin column where the DNA was adsorbed to the silica-gel membrane. Columns were then centrifuged to capture the DNA onto the membrane.

DNA bound to the membrane was washed with two different wash buffers. This step washed through proteins and any other contaminants that may be inhibitory to the PCR by changing pH and varying the salt conditions. The purified DNA was eluted from the QIAamp spin column with AE buffer. The purified fragmented 40 - 50 Kb DNA was then suitable for direct use in the PCR reaction.

### PCR amplification

Guidelines for avoiding PCR contamination were strictly followed (43). For PCR1, DNA was amplified in 50µl volumes containing 10x Qiagen buffer (Qiagen), 3.0mM MgCl<sub>2</sub> (Qiagen), 100µM of each deoxynucleotide triphosphate (Boehringer Mannheim), 0.2µM of primers Cpn1 and Cpn4 (Life Technologies), 1.00 U HotStarTaq™(Qiagen). For PCRn, 1µl of a 1:1000 dilution of the amplified DNA in molecular grade water (Gibco) was re-amplified in 24µl volume containing 10x Qiagen buffer, 2.5mM MgCl<sub>2</sub>, 150µM of each deoxynucleotide triphosphate, 0.2µM of primers Cpn2 and Cpn3, 1.00 U HotStarTaq™.

Amplifications were performed in an automated thermocycler (Perkin Elmer) for both PCR1 and PCRn using 1 cycle of 94°C for 14min, then 38 cycles of 94°C for 1min, 45°C for 1min, 72°C for 1min, with a final extension at 72°C for 10min. The primer sequences used for the PCR are shown in Table 1.

**Table 1. Amplification primers for the 16s rRNA region of *Chlamydia pneumoniae*(1)**

Primer name	Primer sequences	Tm (°C)	Nucleotide position (bp)
Cpn1	5'ATAATGACTTCGGTTGTTAT3'	52	71 - 91
Cpn4	5'TATAAATAGGTTGAGTCAAC3'	52	1448-1468
Cpn 2	5'AGTGAATTAGGCATCTAATAT3'	54	177-199
Cpn3	5'GCTGTATTCTACAGTTG3'	50	1018-1035

### Control of PCR using primers for the human apolipoprotein B-100 gene

An internal control was incorporated within the Chlamydia assay. This primer pair was included as a control against PCR inhibition and to confirm presence of human DNA. The primers amplified a 143 bp product of the human apolipoprotein B-100 gene, which occurs in homo sapiens (EMBL database, Howard Potter, personal communication). Detection of this DNA target sequence indicated that amplification was able to occur due to absence of inhibitors, and also that specimen DNA had been added to the reaction. The primer sequences used for the PCR are shown in Table 2.

**Table 2. Amplification primers for the 16s rRNA region of the human apolipoprotein B-100 gene (Genebank Accession No. M19828)**

Primer name	Primer sequences	Tm (°C)	Nucleotide position (bp)
FDB1	5'CCAACACTTACTTGAATCCAAGAGCACCC3'	65	10550-10580
FDB2	5'TGTACTCCCAGAGGGAATATACGC3'	55	10676-10692

Amplification products were separated by electrophoresis through a 2% agarose gel containing ethidium bromide at 100Vcm<sup>-1</sup> for 10 minutes in 0.5X TBE buffer, with a 100bp DNA molecular weight ladder (Boehringer Mannheim). Target DNA was visualised by fluorescence and photographed.

#### Sensitivity testing

Chlamydial elementary bodies were quantitated for sensitivity testing by estimating inclusion-forming units in tissue culture. Dilutions of ruptured inclusion bodies (obtained by freezing at -80°C for 10min) released individual infectious elementary bodies which were inoculated into host HEp-2 cells in multiple vials and incubated for 2 days at 37°C + 5% CO<sub>2</sub>. The number of inclusions obtained per cover slip was estimated by fluorescent microscopy using an antibody specific for Chlamydia species (Pathfinder, Kallestad, Chaska, USA). The number of inclusion bodies detected was directly related to the initial number of elementary bodies inoculated into the host cell line. A 100µl of suspension of elementary bodies was processed for PCR, the same volume was inoculated into a new vial of HEp-2 cells and incubated to check the concentration of elementary bodies. The cell suspension included the host cell line which is a human cell line and was able to provide target DNA in the first round of amplification for human apolipoprotein, thus acting as a PCR inhibition control.

#### Specificity testing

The primers used were submitted to screening utilising Genebank EMBL database to ascertain whether non-pneumoniae or other bacterial or viral organisms commonly contained sequences which could be detected by these primers.

Other Chlamydial species as well as many other bacterial and viral organisms commonly found in the respiratory system were also tested.

## Results

#### Sensitivity testing: detection of target DNA

Chemical parameters were titrated, and thermocycling and temperature conditions were optimised for the detection *Chlamydia pneumoniae* (44). This resulted in analytical assay sensitivity beyond that achievable by culture. Culture was able to detect 10 elementary bodies/ml while PCR was able to detect one elementary body/ml.

#### Specificity testing

The method employed included a nested step that improved specificity (1). The primers used were submitted for screening to the Genebank EMBL database. This search enabled the detection of any commonly contained sequences between the primers and any other non-pneumoniae strains, and other bacterial or viral organisms. The EMBL BLAST search indicated that the PCR primers were compatible with *Chlamydia pneumoniae* only. Laboratory testing on other Chlamydial spp. confirmed the specificity of these primers.

Other bacterial and viral organisms tested that are commonly encountered in the respiratory system were not detected in clinical specimens by these primers, however any such test panel is always

"incomplete" as it cannot cover all organisms encountered in such specimens.

#### Results of assays

The serology MIF test was compared to the developed molecular assay for the direct detection of *Chlamydia pneumoniae*.

Number of patient specimens tested were 211. Number of control patient specimens tested were 45. Serological detection of *Chlamydia pneumoniae* antibodies detected - 0 positives. Molecular detection of *Chlamydia pneumoniae* DNA detected - 0 positives.

	Test specimens	
	PCR+	PCR-
MIF+	0	0
MIF-	0	211

	Control specimens	
	PCR+	PCR-
MIF+	0	0
MIF-	0	45

Both tests indicate that there was no *Chlamydia pneumoniae* detected in the pilot test population during the specimen collection period. An incidence of 1% would be indicated by an average of 2 patients with positive results in 211 patients. Therefore it is suggested that the incidence of *Chlamydia pneumoniae* is apparently <1% for this pilot study. Due to the lack of any positive results, positive and negative predictive values do not apply.

## Discussion

The purpose of this study was to develop a PCR- based assay for *Chlamydia pneumoniae* pneumonia and to compare that with the MIF serology assay to obtain a statistical profile of infection by *Chlamydia pneumoniae* in a pilot CAP study population. These statistics provide baseline data about the prevalence of *Chlamydia pneumoniae* pneumonia. These results can provide significant data for planning health services and determining whether the incidence of pathogens causing pneumonia is sufficient to justify rapid and specific testing by PCR screening for bacterial pathogens.

The results of this pilot study suggest that the incidence of pneumonia caused by *Chlamydia pneumoniae* was not a major cause of hospital admissions in New Zealand during the study period. However, there are some factors worth considering that may have influenced the numbers of negative results obtained:

## Interpretation of the PCR result

The results of this pilot study are interesting as antibodies to this organism have been reported in previous studies in New Zealand by serology diagnosis (6-8) although none of these results were ever confirmed by another technique. Although just under half of the CAP pneumonia patients were tested by PCR in this pilot study it is possible that there may be patients who are positive in those specimens yet to be tested.

### Assessment of clinical specimens for PCR

The suitability of the sample for the test should also be considered. Throat swabs collected in PBS were used as the respiratory sample source, whereas throat swabs collected in Chlamydia transport media had been used by others (1,3,5). This additional storage media may have assisted in retaining the integrity of the sample DNA. It is possible that the fastidiousness of the organism rendered poor quality DNA that could not be amplified by PCR.

A nested PCR was able to overcome the inhibitory effect of Chlamydia transport medium and bacteria present on the swab (including *Chlamydia pneumoniae*) (1). Additionally, corresponding WBCs were also found to be negative by other investigators (13). It is possible that overwhelming disease may be required before the bacterium is expressed in blood cells.

### Possible adverse affects on the specimens

Administration of antimicrobial therapy before the collection of specimens may have affected bacterial DNA. For pneumonia patients the initial antibiotic administered is penicillin, which is usually effective against *Streptococcus pneumoniae*, the most common cause of bacterial pneumonia (7). The penicillins are not effective against *Chlamydia pneumoniae* pneumonia (14) and therefore this early treatment would not affect the chlamydial DNA. Every effort was made that acute specimens were collected before instigation of antimicrobial therapy.

### In - house PCR assays

Currently there are no commercial assays available for the detection of *Chlamydia pneumoniae* to enable comparison of these results. Usually a commercial system requires a high throughput to be cost effective. Thus our in-house assay was tailored to the laboratory's own needs, dependant upon demand. The choice of primers for the assay is against a region of the 16s rRNA gene, this area is most highly conserved, and exhibited little variation within the species examined (45).

### Analytical sensitivity of the PCR

The PCR had an analytical sensitivity of 10<sup>9</sup> CFU/ml in cell culture growth media comparable to the referenced authors (1). This sensitivity indicated that the PCR could detect organisms in a similar concentration to bacterial numbers present in clinical specimens. Possibly the assay may have a greatly reduced clinical sensitivity in patient specimens and therefore be unable to detect *Chlamydia pneumoniae* in the small amounts as found in carriers. A recent review of five different PCR assays for the detection of *Chlamydia pneumoniae* found that all assays had a similar analytical sensitivity compared to the PCR used for this survey (13), where the sensitivity of all assays was also markedly reduced by the presence of peripheral blood mononuclear cells. The researcher did not confirm this finding as all DNA from white blood cells tested was amplified by the PCR for apolipoprotein B-100.

The sensitivity of the PCR assay utilised defines the incidence of *Chlamydia pneumoniae* within the assayed population. If the sensitivity is low and only able to detect large amounts of DNA then

that assay will mistakenly report a reduced incidence of *Chlamydia pneumoniae* in that population. Conversely an extremely sensitive PCR assay that is able to detect DNA in excess of that encountered in a clinical sample is more prone to detect false positives. Extreme sensitivity of a PCR assay can be obtained by increasing the number of cycles of amplification of the target DNA. However, there seems little point in exceeding the detection level of bacteria found in clinical specimens.

### Limitations of the serological assay

One should be mindful of the following limitations when interpreting the results. Interpretation of the MIF result requires special skills. The intensity of the fluorescence can be subjective with different operators interpreting an endpoint differently, and non-specific fluorescence may make the endpoint difficult to determine. At lower dilutions the serum may exhibit cross-reaction to all three chlamydial species. The MIF test can cross-react with other bacterial species, i.e. *Pseudomonas* spp. Bacterial contamination of serum or reagents can also produce erroneous results (35). The sensitivity of this serological test is unknown and may not be an adequately sensitive assay (34). Other studies have suggested that an "expanded gold standard" is more indicative of the true incidence of *Chlamydia pneumoniae*, where one test is confirmed by at least one other (5).

### Interpretation of the serological response

The MIF test does present some difficulties with interpretation. Firstly, the baseline titre is set as high as 64 in order to minimise the effect of cross reactivity. For pneumonia patients to have a diagnostic rise in titre their convalescent serum has to have a detectable antibody level at 512. Sub-clinical infections may not elicit such a response, or more serious infection may begin with a non-detectable titre of <16 and have a four-fold increase to 128 which would be diagnostic if the high baseline did not have to be observed.

### Assessment of clinical specimens for the MIF test

The MIF test detects only IgG antibodies. The IgG response may be delayed for up to six weeks and the convalescent sample may have been collected before this response was detectable.

### Specimens tested before this pilot study

Previous to pilot CAP study testing, the *Chlamydia pneumoniae* PCR had been utilised in testing two high-risk groups. Group 1 involved 30 asthmatic patients who had a respiratory swab collected in Chlamydia transport media, these specimens had culture and PCR performed. Group 2 involved 10 atherosclerotic plaque tissue specimens collected at post mortem, these specimens had only PCR performed. These specimens were from high-risk patients and were from sites where *Chlamydia pneumoniae* had previously been detected (1,14,20,24). None of these clinical specimens tested positive for *Chlamydia pneumoniae* by PCR. The respiratory swabs were also cultured and found to be negative. Culture for *Chlamydia pneumoniae* has also been carried out in Auckland for some years and they have yet to detect a positive specimen (Mike Brokenshire, personal communication).

However others researchers have also been unable to detect *Chlamydia pneumoniae* in atherosclerotic plaques (25,26). In these studies live viable tissue were used instead of post mortem specimens. Possibly specimen age may have had a deleterious effect on the *Chlamydia pneumoniae* DNA in our investigation. Sampling site or seasonal variation may assist in explaining the variation.



## Conclusions

To gain an understanding of disease incidence in a given population the most appropriate tests should be utilised. One required test involved set up of a new molecular assay for the pilot CAP study. However, part of introducing a new molecular test into the routine laboratory requires comparison with conventional methods. Ideally PCR should have been confirmed by cell culture. An initial trial, sampling a small number of target population, gave no positive results by culture. Subsequently, culture could not be further justified due to the small number of specimens received, making this service uneconomic. Additionally, the fastidiousness and slow growing nature of this organism rendered the isolation of such in the laboratory of little use in reaching an urgent decision in regard to instituting antibiotic therapy.

The only other comparable test available, other than culture, is serology using either the MIF test or ELISA. The MIF test is available in our laboratory for serological diagnosis of *Chlamydia pneumoniae* but has a concerning degree of reported inadequacies (34) which has been highlighted with the introduction of an "expanded gold standard" (5).

### Laboratory assessment

The results of the two detection methods for *Chlamydia pneumoniae* demonstrated no detectable *Chlamydia pneumoniae* in a pilot study of pneumonia patients in the period studied. Perhaps there is seasonal variation, *Chlamydia pneumoniae* occurs in epidemics, as previously reported (17), and perhaps such an epidemic did not occur at the time of the pilot study sample collection.

Limitation of sample population number, may have given a false negative indication of incidence. However, since the submission of this work the Waikato group have tested relevant specimens from the complete CAP study population for *Chlamydia pneumoniae* using a PCR that targets a different region. They were unable to find any positive specimens, this finding has also been found by the completed MIF serology testing. This has confirmed the findings of our initial pilot study and also provides the true incidence of *Chlamydia pneumoniae* in New Zealand in a CAP study population for that period as <1%.

### Clinical assessment

Study factor information must be observed at the outset.

- The classification of a patient to be investigated for pneumonia disease may have included a number who were eventually diagnosed as non-pneumonia patients. Patients were classified as having pneumonia by radiological changes and clinical symptoms, and the microbiological isolation of a pathogen known to cause pneumonia.
- Likewise the concurrent control group, who were age and sex matched to the test group may also have not fitted the criteria of a patient without pneumonia yet still have stayed within the control group.

### Assessment of test used

The PCR used was challenged with a significant number of clinical specimens. No false positives or false negatives (as confirmed by the detection of the internal control) were detected. However, as the specimens assayed contained no detectable *Chlamydia pneumoniae* it is difficult to validate the assay completely. While the positive control was always detected, it is also necessary to include clinical positive samples for validation. This PCR is now available in our laboratory on a limited basis after consultation with a clinical microbiologist and on the understanding that validation has been unable to be completed. PCRs that have not had complete validation are performed without cost to the clinician. The MIF test, which reportedly detects false positives, did not do so in this pilot study.

The objective of the pilot study was to measure the incidence of *Chlamydia pneumoniae* infection in a target population using the most appropriate tests. The assays were performed double-blind and the assessor of the results, the clinical microbiologist, was not aware of the test and control groups when interpreting the MIF results. This minimised bias in result reporting.

The PCR detects infectious bacteria at acute presentation while the serology MIF test detects a retrospective diagnostic rise in antibody titre. While both methods do not measure the same sample or the same bacterial aspect of the disease, their results should confirm the other test, i.e. a positive PCR result should be confirmed by a diagnostic rise in titre therefore being mutually inclusive thus providing an "expanded gold standard result". The absence of detectable infection by either method suggests that no positives were missed.

The previous positive incidence of *Chlamydia pneumoniae* in past CAP studies was diagnosed by serology or culture only, with an incidence of up to 3%. In retrospect the validity of these findings without any confirmatory test needs to be questioned. Performing PCR on stored previous CAP study specimens would have been of interest, particularly on patients who had sero-converted. Unfortunately these patients only had a urine specimen collected and stored that was not a recommended specimen for the detection of *Chlamydia pneumoniae*. Adequate storage of these specimens was also a concern.

The MIF and PCR results, which detected no indication of *Chlamydia pneumoniae* infection in 211 pneumonia patients, suggests the prevalence of this bacterium in people admitted for investigation of pneumonia in the 1998-1999 pilot CAP study to be less than 1%. This incidence of disease is markedly different from that reported overseas of 10-20% in other populations (1, 2).

### Timing of specimens collected / clinical intervention

- There were strict guidelines for specimen sampling for the CAP study, but occasionally these specimens could not always be collected at the desired time and therefore may not always reflect the accurate disease-state of the patient at the time of sampling. Ideally specimens should have been collected on day 1.
- Delay in sampling time may cause overlap with the beginning of administration of antibiotic therapy. Presence of antibiotics may 1. inhibit the growth / isolation / detection of bacteria, 2. have suppressed the immune response to the infection. However, generally therapy that would affect the tests utilised had not been instigated.

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# A POXY THREAT?

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## Abstract

Smallpox has been recognised for over 3,000 years, and was one of the world's most feared infectious diseases until it was eradicated in 1979 following a vaccination program instigated by the World Health Organisation (WHO). Smallpox disease is severe, it has a 30% mortality rate, is easily spread, and there is no effective treatment available. The public health consequences of a smallpox outbreak would be severe. The causative agent of smallpox is Variola virus from the family *Poxviridae*, subfamily *Chordopoxvirinae*, genus *Orthopoxvirus*. Humans are the only known natural hosts of this virus. All current variola virus stocks are held in one of two World Health Organisation Collaborating Centres in USA and Russia. However, some governments believe that variola virus exists in places other than these laboratories, and that there is the potential for deliberate release as an agent of biological warfare and bioterrorism. WHO have recently updated recommendations on primary strategies for the control and containment of smallpox in the event of an outbreak. In New Zealand, the Ministry of Health has made the decision to import 10,000 doses of the smallpox vaccine to be used should this situation arise. This review outlines the smallpox virus, its history, the disease process, prevention, and the role of the laboratory in its diagnosis.

**Key words:** smallpox, Variola virus, Poxviridae, Chordopoxvirinae, Orthopoxvirus, bioterrorism, Vaccinia virus

## Introduction

Smallpox has been recognised for over 3,000 years, and was one of the world's most feared infectious diseases until it was eradicated following a vaccination program instigated by the World Health Organisation (1). We should be concerned today about smallpox because the disease is severe, it has a 30% mortality rate, is easily spread, no effective treatment is available, it may not be extinct, and it has the potential to be used as a biological weapon. Smallpox was probably first used as a biological weapon during the French and Indian wars (1754-1767) by British forces spreading the disease to American Indians by distributing blankets used by smallpox patients. Recent events have raised public health concerns about the potential release of smallpox as an agent of biological warfare and bioterrorism. In New Zealand, the Ministry of Health has made the decision to import 10,000 doses of smallpox vaccine to be used if there is an outbreak (2). This article will overview the smallpox virus, its history, the disease process, the prevention of smallpox, and the role of the laboratory in its diagnosis.

### The smallpox virus

The causative agent of smallpox is Variola virus from the family *Poxviridae*, subfamily *Chordopoxvirinae*, genus *Orthopoxvirus*. Other members of this genus include Cowpox, Monkeypox, and Vaccinia virus. Humans are the only known natural hosts of Variola virus. Poxviruses are large, brick-shaped viruses about 300 x 200nm (about the size of small bacteria). The virion is coated, and has a complex internal structure. A large dsDNA genome 200kbp in size is enclosed in a core that is flanked by 2 lateral bodies. The surface is covered with filamentous proteins, which gives it the appearance of a ball of wool.

The smallpox virus enters the susceptible host through the upper respiratory tract, multiplies locally, and moves via the lymphatics to the regional lymph nodes, where further multiplication occurs. This is followed by entrance into the blood stream leading to primary viremia. Further viral multiplication leads to secondary viremia and invasion of the epidermis, causing skin eruptions characteristic of smallpox (3). Smallpox mortality is caused by overwhelming viremia affecting multiple organs, leading to multiple organ failure.

There are 2 clinical forms of smallpox, variola major, and variola minor. Variola major is the most common form, which is more severe, and has a more extensive rash and higher fever than variola minor. It has a 30% fatality rate. There are four types of variola major smallpox: ordinary, the most frequent type accounting for 90% of cases; modified, a mild type occurring in previously vaccinated individuals; flat; and haemorrhagic, which are both rare and very severe. Variola minor is less common, and produces a much less severe disease than variola major, having a 1% fatality rate (4).

### History

Prior to the introduction of vaccination and disease eradication, smallpox was one of the most devastating infectious diseases known to humanity. For centuries repeated epidemics swept through continents and killed up to 50% of the affected populations.

- **1967.** WHO headquarters, with the goal of achieving global eradication, established a smallpox eradication unit. This was implemented by a strategy of mass vaccination supported by surveillance and containment.
- **1977.** The last naturally occurring nonfatal case was diagnosed in Somalia (5).
- **1978.** A fatal laboratory-acquired case occurred in a research laboratory in Birmingham, England. It killed a medical photographer whose place of work was above the smallpox laboratory. Her mother was also infected but survived. The virologist involved, an expert on the virus, committed suicide (5).
- **1979.** Smallpox was officially declared eradicated by the WHO. At this time, it was agreed that all remaining stocks of smallpox virus were to either be destroyed, or passed to one of two WHO Collaborating Centres; Centres for Disease Control and Prevention (CDC), Atlanta, USA; and the Russian State Centre for Research on Virology and Biotechnology, Koltsovo, Russia. (6). Some governments believe that smallpox virus exists in places other than these laboratories, and could be deliberately released.

### Transmission

A person infected with smallpox virus becomes contagious with the onset of rash, and remains contagious until the last smallpox scab falls off. Smallpox is spread through person-to-person contact via infected droplets or aerosols. Clothing and bedding contaminated with smallpox scabs can also transmit smallpox, although the risk is much lower. In a closed environment, airborne virus can sometimes spread, for example via ventilation systems. (1,4,7).

The number of cases of smallpox that would occur after a deliberate release of the virus has been demonstrated using mathematical modelling. If there were no intervention, based on 10 initial cases, 3 people infected per infectious person, in 6 months the

total number of cases of smallpox would be 2.2 million. With the use of a combined vaccination and quarantine campaign, based on 100 people initially infected, and 3 people infected per infectious person, approximately 4,200 cases would occur, and 365 days would be needed to stop the outbreak (8). The effective use of a combined vaccination and quarantine campaign, would control any outbreak (8). With today's absence of natural disease and vaccination, the global population is very susceptible to smallpox, and some experts have estimated today's rate of transmission to be 10 new infections per infected person (1).

### Smallpox disease

Smallpox disease has an incubation period of 10-14 days, during which there are no symptoms, and a person is not contagious. The incubation period is followed by the sudden onset of influenza-like symptoms lasting for 2-4 days. This is called the prodrome phase. Symptoms include fever, malaise, head and body aches, prostration, and occasionally abdominal pain and vomiting. A person can sometimes be contagious during this period. At the onset of rash, day 1, spots in the mouth develop into sores that break open and spread large amounts of virus into the mouth and throat. At this time, a person is most contagious. The rash starts on the face and spreads to the arms and legs then to the hands and feet within 24hrs, and progresses to the trunk after a few days. As the rash appears, the fever usually falls. By day 3, the rash becomes raised and papular. By the 5th day the papules become vesicular, filling with thick opaque liquid, and become firm to touch. By day 7 the vesicles become pustular, the fluid becoming cloudy and looking like pus. At this stage the lesions are deeply embedded in the skin, and the fever usually returns. By about day 10, the pustules dry up and form scabs. After 3-4 weeks, most scabs will have fallen off, leaving pitted scars. Scabs contain live smallpox virus, so a person is contagious till all the scabs have resolved. (1,4,9).

The most effective measure against smallpox is vaccination before exposure. The smallpox vaccine contains live unattenuated vaccinia virus, another member of the Orthopoxvirus family, derived from cattle suffering from cowpox. Vaccination is performed via intradermal inoculation by multiple puncture with a bifurcated needle. A pustule appears at the vaccination site, and leaves a scar, indicating vaccination. The duration of immunity following vaccination is not known for certain. Protection is not believed to be long lasting. Those considered to be at high risk should be revaccinated annually, and those less likely to come into contact with smallpox every 3 years (10).

### Complications of vaccination

Smallpox vaccination is associated with serious complications, and in extreme cases can be fatal, with approximately one death per million resulting from primary vaccination (1). Four main complications are associated with vaccination; generalised vaccinia, progressive vaccinia, eczema vaccinatum, and post vaccinal encephalitis. Generalised vaccinia is characterised by the development of a generalised rash after vaccination of healthy individuals. Progressive vaccinia occurs in immunocompromised individuals. In these cases, the lesion at the vaccination site fails to heal, and secondary lesions may appear elsewhere. Eczema vaccinatum occurs in individuals suffering from or who have a history of eczema. Vaccination can cause a local or systemic disease in these individuals. Post vaccinal encephalitis is the most serious complication, and has the highest fatality rate. It resembles other types of post infection encephalitis. (4).

There are several groups who have a high risk of suffering from complications after smallpox vaccination; people with eczema, a history of eczema, or other significant skin conditions such as

dermatitis, burns, impetigo, and varicella zoster; the immunosuppressed; pregnant women; and the elderly. (4).

### Treatment

Vaccination in the first 4 days after exposure is effective in reducing the morbidity and mortality of smallpox disease. No effective treatment, other than the management of symptoms is currently available (1). Vaccinia immune globulin (VIG) is a solution of gamma globulin from the serum of recently vaccinated individuals. It can be given concurrently with smallpox vaccine to help prevent adverse reactions in at-risk individuals, and is normally reserved for this purpose (10). The antiviral agent Cidovir has produced promising results in laboratory studies (1). However, since it is nephrotoxic and needs to be administered by injection or intravenously, it is not an ideal drug for the treatment of smallpox. Efforts are being made to identify new compounds active against Variola virus (11).

### The laboratory

Confirmation of Variola virus will be confirmed by one of two WHO Collaborating Centres; Centres for Disease Control and Prevention (CDC), Atlanta, USA; and the Russian State Centre for Research on Virology and Biotechnology, Koltsovo, Russia. (6). These laboratories possess high containment facilities at Biosafety Level 4. Biosafety level 4 facilities are in a separate building, or completely isolated from all other areas. All activities are confined to Class 3 biological safety cabinets, or Class 1 or 2 cabinets used with a one-piece positive-pressure suit ventilated by a life support system. All materials are autoclaved or decontaminated before leaving the facility, and personnel enter and leave via a clothing change and shower room (12). Currently in NZ there are no human biosafety level 4 facilities, so any specimens received for smallpox testing here would be sent to CDC in Atlanta.

Specimens suitable for laboratory diagnosis include vesicle fluid, scrapings, and vesicle crusts. The diagnostic techniques used for the diagnosis of smallpox include, electron microscopy, cell culture and nucleic acid detection. Electron microscopy (EM) involves the negative staining of specimens prepared on an EM grid. EM is the fastest and most dependable diagnostic procedure for poxviruses, however members of the Orthopoxvirus genus cannot be differentiated. For the isolation of Orthopoxviruses, human embryonic lung or primate cell lines are recommended. Variola virus causes cytopathic effect involving cell fusion and multinucleated foci in these cell lines. Following isolation, the specimens are inoculated onto chorioallantoic membranes of chick embryos and incubated. Each orthopox virus will produce characteristic pocks (3). Variola virus produces white, opaque, smooth, dome-shaped pocks and may have a halo giving a fried egg appearance. A number of nucleic acid based methods have been developed to distinguish variola virus DNA from other Orthopoxviruses. These include RFLP analysis, multiplex PCR, and real-time PCR (13). If a variola virus outbreak should occur, the diagnostic laboratories that are currently prepared would easily be overwhelmed, so it is important that detection protocols, diagnostic reagents and training materials are shared with other approved diagnostic facilities (13).

### Bioterrorist attack action plan

In the event of a bioterrorist attack, an action plan is needed to control an outbreak of smallpox. This plan will involve surveillance to identify infected individuals and also to monitor contacts. It will also involve the isolation of infected individuals, who should be isolated immediately, preferably at home, until all scabs have been shed from any lesions. Finally, a ring vaccination strategy needs to be put in place. Since current vaccine supplies are limited, and general mass vaccination

is not recommended, high-risk groups are prioritised. These groups include; people exposed to the initial release of the virus; people who have had close-proximity, or household contact with confirmed or suspected smallpox cases after development of fever until scabs have resolved; direct contact medical or public health personnel; laboratory staff; and people who are essential to the response activities of an outbreak.

At present there is no evidence of illicitly held smallpox virus, and the risk of its deliberate release is likely to be low (4). However, since smallpox disease is severe, has a high mortality rate, and is easily spread, the public health consequences of the deliberate release of smallpox virus would be severe. Primary strategies for the control and containment of smallpox in the event of an outbreak need to be updated and implemented to ensure public health preparedness. Recent WHO recommendations are available from CDC, Atlanta. In New Zealand, the Ministry of Health has made the decision to import 10,000 doses of the smallpox vaccine to be used if there is an outbreak (2). So is smallpox a poxy threat, and are we prepared for the potential deliberate release of Variola virus in New Zealand?

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# A quality assurance programme for health laboratories in the South Pacific

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## Abstract

The Pacific Paramedical Training Centre provides technical training and assists with the transfer of appropriate medical laboratory technology to the Pacific Islands. As a World Health Organisation Collaborating Centre it has an additional responsibility for external quality assurance.

The Pacific Regional External Quality Assurance Programme was started in 1990 with 16 laboratories enrolled and by 2002 the total was 21. Surveys in haematology, biochemistry, microbiology and transfusion medicine were dispatched on a regular basis to participating laboratories.

Overall laboratory participation rate has averaged at 70% (ranging from 62-80%). Biochemistry results have shown a general improvement over time, while transfusion medicine results have been consistently high. For microbiology and haematology the overall trend has, at best, been neutral. A number of factors have impacted negatively on the survey results for some participating laboratories. These are: delays in survey delivery, equipment maintenance, reagent supplies, and financial constraints.

The quality assurance programme has assisted many laboratories of Pacific Island countries to improve the quality and accuracy of their test results.

## Key words

Quality assurance; South Pacific; medical laboratory technology

## Introduction

The Pacific Paramedical Training Centre (PPTC) was established in 1980 for the purpose of providing technical training and to assist with the transfer of appropriate medical laboratory technology to the Pacific Islands. The effectiveness of the PPTC training programmes was recognized by the World Health Organization (WHO) and after consultation with the New Zealand Government in 1990 the Centre was designated a WHO Collaborating Centre with responsibilities for technical training and external quality assurance.

Included in the WHO terms of reference was the task of organizing and conducting external quality assurance programmes in microbiology, haematology, biochemistry and immuno-haematology (transfusion medicine) for health laboratory services of developing countries, especially those in the South Pacific. These programmes were to include training and technical consultation, provision of analytical material, evaluation of results, and feed back systems.

This was the basis on which the Pacific Regional External Quality Assurance Programme was established and the design, implementation and outcomes for the period 1990-2002 are discussed in this paper.

The purpose of Pacific Regional External Quality Assurance Programme was three fold:

- To stimulate the need for technical improvement
- To provide an effective management tool for internal quality control
- To provide an independent and objective performance assessment mechanism

## Methods

At the commencement of the Pacific Regional External Quality Assurance Programme in 1990, 16 laboratories were enrolled, in 1995 one additional laboratory joined, and by 2002 the total was 21. Although the laboratories enrolled in the programme are primarily the national laboratories of the various Pacific Island countries, laboratories from Laos PDR and Vietnam are also involved. The scope of the programme has always been to assess the laboratory's capacity to produce accurate and reproducible results in the medical laboratory science disciplines of haematology, biochemistry, microbiology and transfusion medicine.

Surveys are packaged and labelled according to the relevant IATA regulations and dispatched on a regular basis to the participating laboratories. The laboratories are given one month from the date of dispatch to return their results to the PPTC.

With the exception of biochemistry, one month following dispatch of the survey an immediate response is sent to each laboratory giving the results obtained by the referee laboratory. Following this, the results received from each laboratory are assessed and marked by the co-ordinator responsible for the discipline, and a detailed analysis and critique of the results are returned in a timely fashion.

From 1990-1999 the programme consisted of six to eight surveys/year with up to two surveys in each discipline being sent by courier to each participating laboratory. In 2000 the frequency was increased to 11 surveys/year, three each in haematology, microbiology and transfusion medicine, and two in biochemistry. Each biochemistry survey, however, consists of two sets of material to be analysed one month apart but with results to be sent back as soon as each component has been analysed.

Table 1 lists the countries that participate in the programme. In most instances only the 'national' or central laboratory is registered in the programme. However, there are some exceptions to this, for instance in Fiji, where both Suva and Lautoka participate, and Federated States of Micronesia, where each State's Central Laboratory is a participant.

The programme is provided free of charge to all the central laboratories through a funding grant received from the WHO Regional Office in Manila plus an annual grant from NZAID and a generous donation from the NZIMLS. A brief description of the materials sent and the method of marking for each discipline follows.

**Table 1. List of participating laboratories**

Country	Laboratory	Date Joined
Federated States of Micronesia	Yap State Hospital Laboratory	1990
	Chuuk State Hospital Laboratory	1990
	Pohnpei State Hospital Laboratory	1990
	Kosrae State Hospital Laboratory	1995
Republic of Palau	Belau National Hospital Laboratory, Koror	1990
Solomon Islands	National Referral Hospital Laboratory, Honiara	1990
Niue	Lord Liverpool Hospital Laboratory	1990
Vanuatu	Vila Central Hospital Laboratory, Port Vila	1990
Kiribati	Tungaru Central Hospital Laboratory, Tarawa	1990
Tuvalu	Princess Margaret Hospital Laboratory, Funafuti	1990
Tonga	Vaiola Hospital Laboratory, Nuku'alofa	1990
Samoa	National Laboratory, Dept of Health, Apia	1990
Cook Is	Rarotonga Hospital Laboratory, Rarotonga	1990
Fiji Islands	CWM Hospital Laboratory, Suva	1990
	Lautoka Hospital Laboratory, Lautoka	1990
Republic of the Marshall Islands	Majuro Hospital Laboratory, Majuro	1990
Papua New Guinea	Port Moresby General Hospital Laboratory	1990
	National Analysis Laboratory, Lae	1994
Laos PDR	Mahosot Hospital Laboratory, Vientiane	1991
	Lao Red Cross Blood Transfusion Centre Vientiane.	
	Center for National Laboratory & Epidemiology Vientiane	
Nauru	Republic of Nauru General Hospital Laboratory	1994
Vietnam	Binh Dinh Province General Hospital Laboratory	1998

### Microbiology

Each survey usually consists of three organisms for identification and, if necessary, antimicrobial susceptibility testing. A range of both gram-positive and gram-negative organisms are included and from time to time a more fastidious organism may also be sent. One of the main constraints with sending fastidious organisms such as *Neisseria* and *Haemophilus* species and to a lesser extent *S. pneumoniae* is that from the date of dispatch it may take anywhere from three days to three weeks for the specimen to arrive in the laboratory. All cultures are sent on a solid-media slope because of difficulties some laboratories have in opening and reconstituting lyophilised vials in a safe manner.

When results are returned to the PPTC they are checked and marks allocated for identification and susceptibility testing. Because of lack of resources in some laboratories, marks are not necessarily allocated just for the correct answer, as at times a laboratory may not have the reagents or media to fully speciate an organism. Therefore emphasis and marks are allocated for tests performed and how the laboratory arrived at submitted results.

### Infectious diseases serology

Several times a year a serum sample will be included with the microbiology survey for the laboratory to perform the tests they would use in screening a potential blood donor. Normally this would include Hepatitis B surface antigen, a test for syphilis, and also for HIV 1 & 2. A small number of laboratories are also testing for Hepatitis C antibodies.

### Haematology

This programme is composed principally of stained blood smears. In each survey a number of smears stained by May Grunwald /Giemsa Romanowsky stain are sent for examination. From 2001 a lysate, kindly provided by NEQAS (UK), has been included for each laboratory to

perform haemoglobin, white cell count and platelet count. As an alternative to stained smears, the last survey in 2001 included five photomicrographs of various blood film abnormalities. The purpose of sending these, rather than stained smears, was that each laboratory would be able to commence a collection of photographs with complete explanation of the disorder which could then be used as a staff training tool. Each smear or photograph is accompanied by a detailed resume of the patient's clinical status, as well as instructions as to how it must be dealt with in terms of observation and reporting.

When the referee laboratory receives the results they are evaluated according to a marking schedule approved by WHO. This results in a final % mark that is reported back to the laboratory. The referee also provides written discussion concerning each answer given, which is designed to be both informative and constructive. Each laboratory also receives a reference evaluation for each case study included in the survey, which is considered educational in nature, supplying not only details of correct film findings and diagnosis but also clinical and laboratory information that will be useful for future reference.

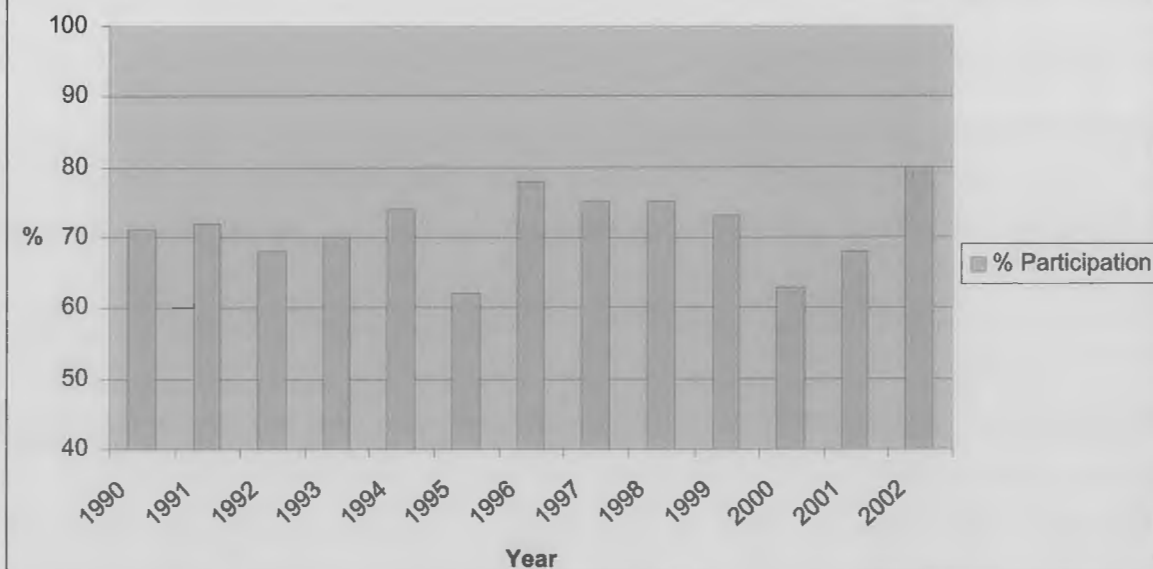
### Transfusion medicine

In this programme each survey consists of blood and serum from a 'recipient' and three samples of red cells from possible 'donors'. The laboratory is required to carry out blood grouping (ABO and Rh) on each sample and cross match the donor's samples with those of the patients. From time to time antibodies are included to ensure some donors are incompatible. During 2002 the surveys included requests for protocols that cover the provision of blood in various scenarios, including emergencies.

When assessing returned results, marks are allocated for correct forward grouping [anti-A, anti-B and anti-A+B], reverse grouping Rh(D) typing, and interpretation of results. As red cell antibody screening [RCAS] is not performed by many of the participating laboratories, it is



## Laboratory Participation Rate



expected that crossmatch methods will include room temperature (RT) and IAT techniques, or other similar techniques, such as albumin. Marks are allocated according to the methods used and correct results.

A few laboratories are able to screen and identify antibodies and marks are allocated for this. But if a laboratory does not have the capacity to perform these tests, they are not penalized. The laboratory is requested to supply information on the reagents used and their expiry dates, and marks may also be allocated if this information is supplied.

### Biochemistry

This programme consists of two shipments/year and we are indebted to the RCPA-AACB Chemical Pathology Quality Assurance Group for supplying us with the control material and its accompanying target values. In more recent years they have also given us statistics for different instruments, which has been enormously useful in recognising instrument bias.

Each shipment contains four samples; two to be analysed in the month of delivery and two a month later. An interim report is sent when the first pair of results are received at the PPTC. This report gives information on the participants results compared to the acceptable range, and comments on linearity, accuracy and precision. Where possible results are compared with RCPA-AACB statistics from similar methods. A final report with the % results in range score and an accompanying explanatory letter are sent on receipt of all results.

## Results

The overall laboratory participation rate for the 11 years has averaged at 70% with a peak of 80% in 2002 and a trough of 62% in 1995 (Figure 1). Because there is no compulsion for laboratories to participate in the programme the response rate appears to peak and then gradually fall off until a reminder is sent to low participation rate laboratories, reminding them of the advantages to both them and their country's health services of active participation. A number of laboratories have been consistently good participants while others have been inconsistent. Other factors that impact on participation are discussed later.

When reviewing results, only those from biochemistry and transfusion science fit the requirements for statistical analysis, as they are the only two where there has been relative stability in the type of samples sent and the procedures used for marking returned survey results.

Biochemistry results as a whole have shown a positive trend that indicates an improved accuracy in analysis over the 11 years of the surveyed period. Individually, nine laboratories that participated in the biochemistry QA programme in 1990 and also participated in the 1<sup>st</sup> round of 2001, showed a statistically significant improvement. Mean % scores (with 95% confidence intervals) for 1990 and for 2001 were 48.9% (35.4-62.4) and 63.4% (52.1-61.7) respectively ( $p=0.048$ ; paired t-test).

For transfusion medicine, apart from a few individual laboratories, % survey were consistently high. Ten laboratories participating in this survey in 1990 that also participated in the 1<sup>st</sup> round of 2002 returned mean % scores (with 95% confidence intervals) of 81.9% (70.3-93.5) and 80.0% (69.8-90.2) respectively ( $p=0.693$ ; paired t-test).

For microbiology and haematology the overall trend has, at best, been neutral. The results from these disciplines could not be analysed statistically for the reasons given above, but within individual surveys and disciplines the swing in marks have been quite significant. In addition, although there may not be an overall improvement, positive trends have been seen within individual laboratories when comparing their results over the period in which they have participated. There are a number of factors that contribute towards this seeming lack of improvement and these will be discussed later.

For haematology the identification of blood film abnormalities and general quality of result reporting is continuing to improve at a steady rate and this is a direct result of the quality education provided by the PPTC through ongoing survey work, as well as morphology courses run by the Centre, both in New Zealand and in the home laboratory. Strong emphasis is now being placed on interpretation of results and how to report results that are significant and meaningful to the clinician.

## Discussion

Although statistical analysis of the results of the Pacific Regional

External Quality Assurance Programme may not have shown much overall improvement, and in some areas no improvement at all over the 12 years the programme has been running, the positive benefit to many laboratories on an individual basis is immeasurable. As shown in the results section, individual laboratories have shown a statistically significant improvement in biochemistry during this period while in other disciplines, that could not be analysed statistically, an observable improvement has been seen in a number of laboratories.

A number of factors have been identified that have impacted negatively on the survey results for some of the participating laboratories during the period that is being reviewed.

- **Delays in delivery of surveys.** The courier sends all survey samples in the programme and the time from dispatch from the PPTC to receipt in the recipient laboratory may vary from three days to four weeks. The average delivery time for the last four surveys dispatched has been from 11 to 15 days. As in many instances there are no refrigeration facilities available for storage of the samples during transportation and the time it takes for samples to reach the recipient laboratory, deterioration of samples can occur. This may include microorganisms becoming non viable, whole blood samples for grouping and crossmatching lysing, and samples for biochemistry analyte analysis deteriorating due to prolonged exposure to high temperatures. Discussions are ongoing with the courier to try and reduce these delivery delays, but because of the geography of the region, the infrequency of flights to some countries, and the lack of involvement of the larger international couriers in servicing the region, future improvements seem to be in doubt.

- **Equipment maintenance and supplies.** One of the major factors that influence laboratories participation in the Pacific Regional External Quality Assurance Programme is the availability of reagents and, to a lesser extent, equipment breakdowns. Many laboratories in the Pacific Region do not have reagent supply contracts and hence reagents are purchased on an 'as needed' basis. Because of delays in processing orders and making payments, it is not unusual for a laboratory to run out of reagents and therefore not being able to do particular tests. This applies especially in biochemistry and to a lesser extent in microbiology. In addition, only a small number of laboratories have instrument service contracts and hence, when an instrument breaks down, it may be out-of-action for many weeks and possibly months before finance is found for repairs. The effect of this is that an individual laboratory may participate in a particular survey but is then unable to participate in the next few cycles of that same survey. The range of tests that the laboratory can perform may also vary from survey to survey depending on availability of reagents. As instruments do not have regular servicing this can result in a 'fall off' in the accuracy of test results.

- **Financial constraints.** Many laboratories participating in this programme are in countries where fiscal constraints are in place. This can have a major impact on their ability to purchase reagents and hence from time to time, if they want to do a particular test, out-dated reagents will be used. Putting aside the question of legal responsibility, the use of out-dated reagents may give inaccurate results.

These are the main factors that can adversely influence the participation of a laboratory in the programme and also the accuracy of the results they submit.

Over the 12 years of the programme, a number of laboratories have acquired new biochemistry analysers. This acquisition is the one identifiable factor that has led to an improvement in the accuracy of results. Most laboratories that have purchased new analysers have at

the same time entered into both reagent supply contracts and planned maintenance programmes with the supplier. Indications are that accuracy improvements have been maintained as a result of these contracts being introduced and the additional staff training on the instrumentation may also have added to this maintenance of accuracy.

To improve the quality and accuracy of results being produced by many of the laboratories participating in the PPTC's Pacific Regional External Quality Assurance Programme, a multi-pronged approach is required. As well as the Pacific Regional External Quality Assurance Programme, the PPTC conducts several short-term courses each year. These courses cover both theoretical and practical aspects of medical laboratory sciences. Over the years several hundred laboratory technicians from the Pacific Island countries have attended these courses and although it cannot be quantified, indications are that an overall improvement in skills and standards has occurred as a result of this training, and thus this is one prong that needs to be continued if improvement is to be maintained.

A second prong is the requirement for training to improve general laboratory management skills. This is needed so that improvements in activities, such as stock management, planned equipment maintenance, and budgeting can be made in an attempt to minimize problems related to lack of reagents and equipment breakdowns, all of which have adverse effects on participation rates and marks obtained in the Pacific Regional External Quality Assurance Programme.

The third prong to improving quality is for a laboratory to remain involved in a quality assurance programme, as being able to compare themselves with their peers can be an incentive to at least maintain and preferably improve their results.

Finally, we believe that it is the responsibility of the quality assurance programme provider to supply surveys that are relevant to the level of competency of the laboratories, and to ensure that there is a strong educational facet to a programme. A major emphasis of the PPTC's Pacific Regional External Quality Assurance Programme is this educational aspect. Each set of results returned to participating laboratories includes educational material relevant to the topic covered in the survey, and includes information to assist laboratory staff in improving the quality of their results.

The data presented here indicates that the PPTC's Pacific Regional External Quality Assurance Programme has been instrumental in assisting many of the laboratories in Pacific Island countries to improve the quality and accuracy of their test results. It also indicates, however, that there is still some way to go before all laboratories have reached a satisfactory level of accuracy which can be maintained, and therefore the programme must be ongoing to ensure that assistance is given to these laboratories to assist them attain this acceptable level.

## Acknowledgements

The authors wish to acknowledge the assistance of RCPA-QAP Pty Ltd., NEQAS (UK) Haematology Programme, New Zealand Ministry of Foreign Affairs and Trade, the NZIMLS, WHO and Capital and Coast District Health Board Laboratory for their contributions towards the running of the Pacific Regional External Quality Assurance Programme. We also wish to acknowledge all of the laboratory staff that participate in the programme and the positive feedback we receive from them.

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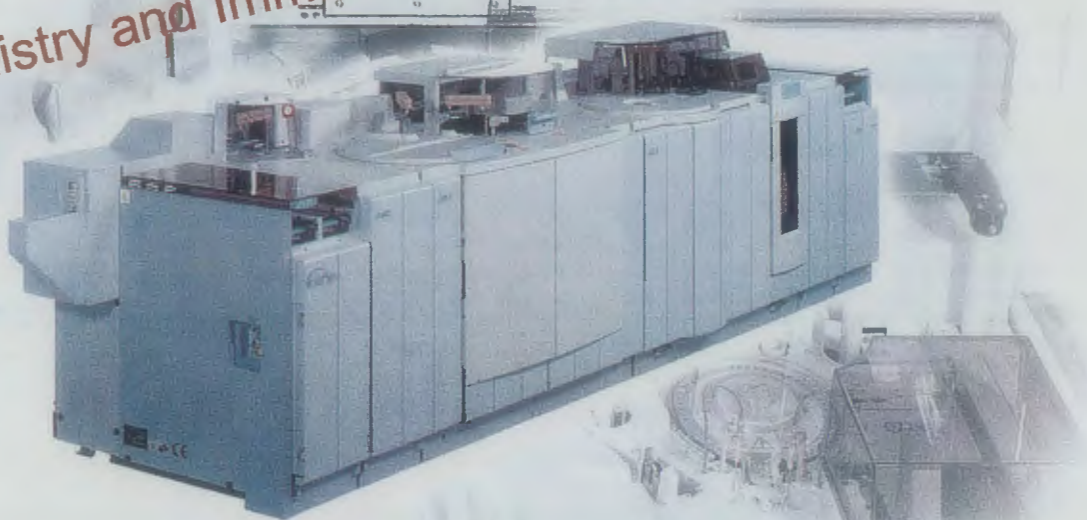


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# Comparison of the RELISA®ENA multi-parameter antibody screening test kit and INNO-LIA ANA Update kit for detection of antibodies to extractable nuclear antigens

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Any malfunction of immunological mechanisms can cause the immune system being triggered by autoantigens, which are host proteins, leading to autoimmune diseases (1). The pathology of autoimmune diseases is inflammatory tissue damage and symptoms such as fever, fatigue and malaise (2). Autoimmune diseases occur far more frequently in women than in men, and the role of genetic factors on the incidence, onset or type of the autoimmune disease is clearly evident (3). Since the production of high-affinity circulating autoantibodies is another characteristic of autoimmune diseases, continuous research in autoantibodies is important in diagnosis and/or confirmation of such diseases. The incidence of autoantibodies in the general population rise steadily with age, peaking at 70 years.

Extractable nuclear antigens (ENAs) are nuclear antigens that can be extracted from the nucleus by chemical means (4). ENAs are a heterogenous group of ribonucleoproteins and non-histone proteins with different functions in nuclear metabolism (5). Detection of autoantibodies to ENAs are important because of its use in the diagnosis and prognosis of autoimmune diseases (4) which affect 5-7% of the human population, often causing chronic debilitating illness (2). The most relevant ENAs are anti-SS-A, anti-SS-B, anti-Sm, anti-RNP, anti-Scl-70, anti-PCNA and anti-PM-SCL. Anti-Jo-1 is a cytoplasmic antibody but is often considered as an ENA.

Antibodies to ENAs are found in patients' blood samples and there seems to be a connection between the type of ENAs antibodies and particular autoimmune diseases (1,5):

- antibodies against Sm antigen and protein-RNA complexes called ribonucleoprotein (RNP) with systemic lupus erythematosus (SLE)
- antibodies against RNP antigens and mixed connective tissue disease (MCTD)
- antibodies against SS antigens and Sjogren's syndrome (SjS)
- antibodies against Scl-70 antigen and scleroderma
- Jo-1 and polymyositis (PM) or dermatomyositis (DM)

Two kit sets, RELISA(r)ENA multiparameter antibody screening test kit (Immunoconcepts) and INNO-LIA ANA Update kit (Innogenetics) were tested for detection of antibodies to ENAs. This study was part of a 'method comparison and critique assignment' required for course completion for fourth year Advanced Clinical Biochemistry and Immunology for the Bachelor of Medical Laboratory Science degree at Massey University.

Thirty-six consecutive serum samples accumulated over the period of a few weeks were screened with the ENA Screen Microplate Kit (Kallestad Diagnostics) on a BioRad CODA EIA analyser. Nineteen serum samples with the highest concentration of antibodies to ENA in the screening test were chosen for comparison. Procedures recommended by the two manufacturers were followed. Results are shown in the Table below.

From the result it was not possible to conclude which kitset was better and results were more or less consistent overall. However, the INNO-LIA ANA Update kit was more labour intensive. Screening 20 samples took 6-7hr, compared to 3-4hr for the RELISA® ENA multiparameter antibody screening test kit. Cost of screening one sample with the Kallestad ENA Screen was about NZ\$6.00, about NZ\$45.00 for the RELISA®ENA multiparameter antibody screening test kit and about NZ\$56.00 for the INNO-LIA Update kit. There were some limitations to this study in that only a small number of positive samples were compared and the effect of repeated freezing/thawing of the samples was not investigated.

There was no attempt to make a provisional diagnosis from the results in this study, as that requires knowledge of appropriate clinical details together with other related laboratory results. Clinical and laboratory findings in relation to the detection of antibodies to ENAs are important because some individuals have high titres of ENA antibody with little or no evidence of clinical disease, whereas some patients with systemic rheumatoid arthritis may have undetectable levels. Other findings may help because there is a discrepancy in

Sample No.	Kallestad ENA Screen Kit	RELISA®ENA multiparameter antibody screening test kit	INNO-LIA ANA Update kit
1-10	None	None	None
11	None	RNP and possibly SS-A, SS-B and Jo-1	Invalid result: control did not work
12	SS-A, RNP	RNP, SS-A and possibly SS-B	Very weak RNP-C
13	Scl-70	Scl-70	Very weak Ro52 (SS-A)
14	SS-A	SS-A and SS-B	Scl-70
15	None	SS-A, and possibly Scl-70	Scl-70
16	SS-A	SS-A	Very weak Ro52 (SS-A), Ro60 (SS-A)
17	SS-A and SS-B	SS-A and SS-B	Ro52, Ro60, SS-B
18	RNP and SS-A	RNP, SS-A and possibly SS-B	RNP-70k, RNP-A
19	Scl-70	Scl-70	Scl-70

diversity of antibodies present in different diseases, antibodies being present in more than one autoimmune diseases, different frequency of antibodies in different individuals for a particular autoimmune disease and lastly, different reactions between autoantibodies to a particular ENA in different diseases (1). There is also known significant differences in EIA kits from different manufacturers and differences between kits - for example, different buffers affect antibody binding, different antigen epitopes are present, differences in antigen coating concentrations, difference in positive-negative cut-off values, or presence of other contaminating antigens in some of the antigen preparations (3). However, detection of high titres of antibodies to any ENA is diagnostic of systemic rheumatic diseases.

## Acknowledgements

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# Citation of articles from the NZ Journal of Medical Laboratory Science in international biomedical journals

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The New Zealand Journal of Medical Laboratory Science has been a peer-reviewed publication since its start as the Journal of the New Zealand Association of Bacteriologists in 1946, changing its title to the New Zealand Journal of Medical Laboratory Technology in 1960 and to its present title in 1991. Over the years members of the profession have predominantly contributed articles reflecting local topics of interest. Over many years the Journal has been abstracted by a variety of international databases, but never by the main ones, namely Medline and ISI Web of Knowledge™. Thus articles in the Journal would not in general attract citations in international biomedical journals covered by those databases. The Journal is currently, and has been previously, indexed by other international databases such as CINAHL (Cumulative Index to Nursing and Allied Health Literature), EMBASE, and the Australian Medical Index. Therefore, the Journal's articles are accessible to the international biomedical community and may attract citation, if deemed of importance. The purpose of this study was to determine citation of the Journal's articles by international biomedical journals.

The ISI Web of Science™, which covers about 8,500 high impact research journals in the world, was searched for articles that cited the New Zealand Journal of Medical Laboratory Science (or Technology) between 1987 and 2003. Various abbreviations of the Journal were entered, as it is known that errors in journal titles occur frequently in biomedical journals (1).

From January 1987 (when the ISI Web of Science™ introduced cited reference searching) to May 2003, 43 articles published in the journal between 1971 and 2000 were cited 105 times. The average number of citations/article was 2.44, ranging from 1 to 9 citations/article. Eight of the 43 articles were cited five times or more. Of these, five articles were cited five times each (2-6), one article six times (7), one article seven times (8), and one article nine times (9).

It has been estimated that the average number of citations a peer-reviewed article is cited in the biomedical literature in its lifetime is less than once, and if an article is cited five times or more, that article is deemed to be of some importance. Indeed, many articles do not attract citations at all. Generally, because of their nature, case studies and methodological articles attract more citations than other articles, with the exception of articles presenting novel new findings. The articles in the Journal that attracted 5 or more citations (2-9) were generally case studies and methodological articles.

Although the Journal's articles cited were from the seventies and eighties, some continue to attract citation in the 21<sup>st</sup> century. Indeed, one citation occurred as recently as in April 2003 (10). The Journal item that was cited in this 2003 article in the *Journal of Clinical Pathology* was an abstract published in the Journal in 1989 from a presentation by Jean Henry of the Wellington Hospital Microbiology Department at the NZIMLT Wellington Branch Seminar, which won her the Watson Victor prize for best paper presentation (11). In total, this abstract has attracted 3 citations in the international biomedical literature.

In conclusion, despite the Journal not being abstracted by the two major international databases, Medline and ISI Web of Knowledge™, articles published by the profession in their own Journal are being recognised as contributing to the international biomedical literature, as

evidenced by the citations 43 articles published between 1971 and 2000 have attracted.

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# Web Page

## Internet Sites for Medical Scientists

This is the second attempt at providing Internet sites of interest for NZIMLS members. I trust you will visit some of these sites and share any others you find interesting with the readers of this journal.

I have not had any feedback about the first list of sites, so this is your opportunity to let me know about items you would like sites for or your favourites that you would like to share with others.

### Topic

#### Management

1. Articles on a variety of management topics
2. More diverse management articles.

#### Stress

3. Stress related articles.
4. More of the same.
5. Canadian site with tons of relevant info.

#### Medical Newsletters

6. Medical site with good newsletters.
7. More of the same.

#### Bioterrorism

8. Great site with tons of bioterrorism news.

#### SARS

9. NZ Ministry of Health.
10. World Health Organisation.
11. CDC  
[www.cdc.gov/ndidod/sars](http://www.cdc.gov/ndidod/sars)
12. International Society for Infectious Diseases.

#### General Medical

11. Animal parasitology.  
[www.ksu.edu/parasitology/625tutorials/index.html](http://www.ksu.edu/parasitology/625tutorials/index.html)
12. College of American Pathologists.
13. Emedicine adult.  
[www.emedicine.com/med/topiclist.htm](http://www.emedicine.com/med/topiclist.htm)
14. Emedicine paediatric.
15. Lab tests on line.

### Website

[www.refresh.com/ceo.html](http://www.refresh.com/ceo.html)  
[www.pbviews.com/resources/index.asp](http://www.pbviews.com/resources/index.asp)

[www.mindtools.com](http://www.mindtools.com)  
[www.cdc.gov/niosh/sresswk.html](http://www.cdc.gov/niosh/sresswk.html)  
[www.ccohs.ca/oshanswers/pyschosocial/stress/html](http://www.ccohs.ca/oshanswers/pyschosocial/stress/html)

[www.questdiagnostics.com](http://www.questdiagnostics.com)  
[www.webmd.com](http://www.webmd.com)

[www.1st-spot.net/topic\\_bioterrorism.html](http://www.1st-spot.net/topic_bioterrorism.html)

[www.moh.govt.nz/sars](http://www.moh.govt.nz/sars)  
[www.who.int/csr/sars/en](http://www.who.int/csr/sars/en)

[www.promedmail.org](http://www.promedmail.org)

[www.cap.org/CAPToday/captoday.html](http://www.cap.org/CAPToday/captoday.html)

[www.emedicine.com/ped/topiclist.htm](http://www.emedicine.com/ped/topiclist.htm)  
[www.labtestsonline.org](http://www.labtestsonline.org)

Well, this is my lot. Enjoy.  
Feedback to: [graemeb@medlab.co.nz](mailto:graemeb@medlab.co.nz)

**Graeme Broad DMLS Dip Bs(Dist)**  
**Projects Manager**  
**Medlab Hamilton.**





## Obituary

**Graham Lindsay Cameron**  
**15 November 1933 -**  
**20 April 2003**

On behalf of the Laboratory staff, past and present, from the Auckland region, I would like to offer my sympathy to Yvonne, Ian, Andrew, and Amanda and to the extended family. You were all such an important part of Graham's life.

Graham began his association with Medical Technology as a trainee at Auckland Hospital in 1951 under Doug Whillans, as Charge Technologist. To his surprise he discovered that in a total staff of around 35, two of his old Mt Albert Grammar school classmates - Rod Kennedy and Clive Horne were also new trainees and Des Phillip, also a MAGS old boy that Graham had known for many years, was several years ahead.

In those early days Auckland was the Central Lab and the smaller outposts of Middlemore, Cornwall (later to be renamed National Womens Hospital) and Green Lane Hospitals were largely staffed by rotation.

After initial training, where Graham recalls Davida MacKenzie introduced him to the delights of cell counting and brass polishing in Haematology, Graham's first venture was to Green Lane. He rode there by pushbike, his first purchase from his 30 shilling-a-week salary. He gained more experience and knowledge, upgraded to an Ariel motorbike and by 1954 had passed the Intermediate examinations. In his own words he described these first three years in the lab as pretty groovy.

He was voted onto Council as a junior member of the then named Association of Bacteriologists - which today is known as the NZ Institute of Medical Laboratory Science.

This was a time of new discoveries and rapid technical development. Upon qualifying in 1956 Graham was almost immediately put in charge of the Blood Bank under Dr Jock Staveley. He made his mark in the Haematology textbooks with the discovery of the presence of the blood group P antigen in hydatid cyst fluid and jointly published papers with Jock on their findings.

In 1958 Graham took up the position of sole charge of the North Shore Hospital Lab where he was to stay for the next 10 years. This was the same year that Yvonne and Graham were engaged - Yvonne having first caught his eye when she was a new lab recruit in 1954.

At North Shore, Graham developed a friendship with the charge radiographer - Harry Wadsworth. Harry's wife Jan, also a technologist and Yvonne were later to work for many years together at Auckland Hospital as the two families grew.

As the anticipated establishment of the North Shore General Hospital seemed no closer, Graham felt it was time to move on. So in 1968 Graham applied for and attained the position of Charge Tech of Bacteriology at Auckland Hospital. To consolidate

his Microbiology knowledge he also took a position of part-time tutor at ATI under Roly Page, which forced him to read widely and helped to increase his general knowledge of Microbiology.

He continued his enthusiasm for teaching and training often being called upon to be an examiner or moderator for the Medical Technologists Board or Institute and contributing to the development of the diploma and degree courses for technologists at the AUT.

During his 29 years at Auckland Hospital enormous changes occurred. It was at this time that the relationship between Graham and Dr David Bremner was established. Clinical Microbiology saw the proliferation and increased power of antibiotics and the increasingly sophisticated methods for monitoring their use. New and potentially dangerous organisms appeared. Old organisms became new pathogens because of the increased susceptibility of very sick patients and increasingly invasive treatments. Microbiology adopted high tech automated analysers. Commercial kit sets for organism identification became commonplace and the pen was replaced to a large extent by computer technology.

Hospital Management became more focused on funding and costs. Having seen the growth of the outposts in the 60's and 70's - during the 80's and 90's Graham was to oversee the consolidation of the Auckland and Princess Mary (Childrens' Hospital) Laboratories. When he retired in 1997 the amalgamation of Auckland and Green Lane/ National Womens laboratories was underway with the prospect of a further move to a purpose built Laboratory building on the horizon.

Graham was always a leader in these innovations. He was often first to trial new technology. He developed a particular interest in parasitology and antibiotics and was always a great source of knowledge to lab and Clinical staff. He encouraged the establishment of Auckland Hospital as the National Reference Centre for anaerobic organisms lead by Jan Garner and latterly, under Dinah Parr, also the National Mycology Reference Centre.

We who worked with him most recently remember him with great affection and respect. On the one hand he worked diligently and held high standards. Yet he made a truly memorable Santa, accompanied by an assortment of elves and fairies, from the technical and medical staff. At his retirement dinner he arrived resplendent in the Cameron tartan.

He continued to keep in touch with us all. He was one of the first visitors to the new LabPlus. He attended the Microbiology Colony Meeting held just last month. He had a keen wit and amused us with the tales from his travels abroad with Yvonne. He had a passion for limericks and with due respect to any English scholars here today I would like to end with a tribute to Graham:

*Graham, well known for his prose,  
And here, he now lies in repose.  
Could he be sifting sand on a beach,  
Or perhaps with cocktail in reach,  
A fine life, reached its end, I propose!*

A true gentleman at peace

**Contributed by Marie Gillies**

## Book Review

**Microscopic Haematology. A practical guide for the Laboratory (2nd Edition, 2003) by Gillian Rozenberg. Published by Martin Dunitz Ltd., London.**

**Ordering information: Blackwell Publishing Asia, PO Box 378, Carlton South, Victoria 3053, Australia. Cost (GST incl.): A\$135.30.**

Gillian Rozenberg is senior medical scientist at the Department of Haematology, Prince of Wales Hospital, Sydney with over 25 years experience in blood film morphology and has lectured extensively in Australia. She held a successful workshop in New Zealand in conjunction with the 2002 NZIMLS Conference in Wellington and will be conducting other morphology workshops at the 2003 South Pacific Congress to be held on the Gold Coast, October 2003.

Microscopic Haematology is designed for use beside the microscope and has 360 colour illustrations of both rare and common disorders. This second edition has been expanded to include a section on Paediatric Haematology, which is divided into benign, malignant and hereditary disorders. The other feature of the 'Second Edition' is the use of the WHO Classification for neoplastic diseases.

Included with the malignant disorders is a listing of the common cytogenetic findings and the immunophenotypic features. There is a

concise and useful section on the Cytochemical reactions found in Myeloid and Lymphoid specific stains which would be useful to students and those new to blood film examinations. The Glossary at the front of the book explains the abbreviations used with the text and makes for easy reference.

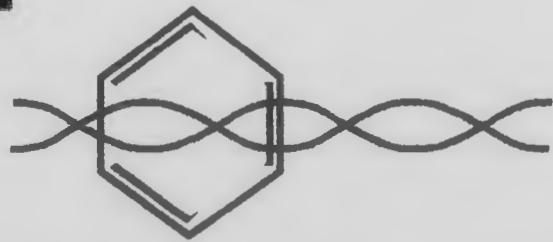
This book is very clearly set out and is broken into four main sections - Erythrocytes, Leucocytes and Platelets, Paediatric and Blood Parasites. Each section contains a brief description of the disorder followed by the blood film findings. The colour illustrations are predominantly peripheral blood images and are grouped at the end of each section. This grouping of illustrations, while different from the usual morphology atlas format, does allow the reader to compare the morphologies such as for Haemolytic Anaemias and Haemoglobin disorders. The quality of the colour illustrations is of a high standard and is truly representative of a well stained blood film.

Overall this book would be a useful addition to any Haematology Laboratory and will be especially useful for students, newly qualified Medical Laboratory Scientists and those wanting "up to the minute" information in an easily referenced format.

*Reviewed by Sue Webber, Senior Medical Laboratory Scientist, Haematology Laboratory, Waikato Hospital, Hamilton.*

## Biochemistry

### Special Interest Group



#### **BSIG meeting held at Novotel Tainui, Hamilton, 10th May 2003**

The biochemistry special interest group met on Saturday 10/05/03 at Hamilton with a smaller number than normal being registered and due to the great Hamilton fog closing the airport for a few hours, thereby delaying some of our southern cousins taking a detour to Auckland. The 65 who attended were treated to some fine first time speakers who did an excellent job of having the courage to stand up and deliver. In all there were 13 speakers who spoke from powdered urine to a Chinese quiz with some excellent talks on instrumentation, QC, problems and what do I do, a trip to the stars and back and of course the winning talk on Experiences with ISO 15189 from Helena Woods from Wellington Pathology. Helena gave an impressive talk on what we new then what we thought we knew and then what IANZ knows. Helena was awarded the Abbott prize for the best-proffered paper. The structure of the committee has now changed with Trevor Walmsley the convener and the members are, Russell Sargon, Helena Woods and Nichola Thomas. The evening started with a wonderful BBQ of steak and sausages and of course the usual lubricant to ease the parched throats of those who attended. It eventually ended sometime Sunday morning with a few stragglers seen walking down the main street of Hamilton on their way to early church! I would personally like to thank the people who attended, the people who gave up their time to

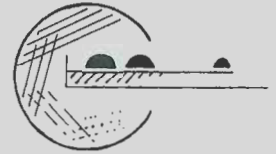
present especially those who spoke for the first time and sponsors, Beckman-Coulter, Roche, Bayer and Abbott. I would also like to encourage those who attended to still consider the directives of the syllabus and examiners who are required. Thanks to a great venue where we were looked after very well.

*Tony Mace, MNZIMLS*



# Microbiology

Special Interest Group



## Abstracts of presentations at the Microbiology SIG meeting, Palmerston North, May 2003

### HPCA update

**Bruce Dove.**

Diagnostic Medlab

The Health Practitioners Competence Assurance Bill's principal purpose is to protect the health and safety of members of the public by providing for mechanisms to ensure that health practitioners are competent and fit to practise their professions. The Bill will empower registration boards such as the MLTB, set to become the Medical Laboratory Scientists (Science) Board, to assess the qualifications and experience of medical laboratory scientists and register them in appropriate scopes of practice.

All staff working in the scope of medical laboratory science will need to be registered.

The MLTB proposes three classes of registration:

- Medical Laboratory Scientists (currently Medical Laboratory Technologists)
- Medical Laboratory Technicians (currently Laboratory Assistants)
- Scientific Officers who are engaged in patient laboratory testing

It is proposed that the basic scopes of practice in medical laboratory science should be defined as the currently recognised disciplines within the profession e.g. Microbiology, Haematology, Cytology, etc.

An authority may register an applicant as a health practitioner permitted to practise within the scope of practice of the profession concerned if the applicant-

- is fit for registration; and
- has the qualifications that are prescribed for that scope of practice; and
- is competent to practise within that scope of practise

The Bill repeals 11 existing regulatory statutes including the Medical Auxiliaries Act 1966 (medical laboratory technologists). The Government is committed to improving the processes for complaints against health practitioners to ensure that they can be resolved expeditiously and fairly with adequate communication between the various agencies involved.

### An uncommon cause of vulvovaginitis in pre-pubertal girls

**Dell Melling.**

Diagnostic Medlab

Vulvovaginitis in pre-pubertal girls (under 12 years) is the most common gynaecological complaint seen in paediatric practice. This age group is particularly at risk because physically they have fewer barriers to infection, and biochemically their vaginas have a pH of around 7.0. The predominant flora is anaerobic bacilli and peptostreptococci. At puberty, estrogens kick in to lower the pH to the normal adult range of  $4.2 \pm 0.3$  and lactobacilli can then colonise.

Vulvovaginitis can be caused by poor hygiene, tight clothing, using perfumes, soaps, foreign bodies, parasite infestation or bacterial infection, including *Streptococcus pyogenes*, *Staphylococcus aureus*, *Candida albicans* and sexually transmitted bacteria.

In January DML received a vulval skin swab from a 6-year-old female. In our routine culture of this swab we included an XLD plate as part of a study. After 24 hours incubation the XLD showed a mixed population of gram-negative bacilli. It is worth noting that if the XLD had not been

inoculated, the GNB's may have been overlooked. A faecal pathogen screen was done and an API 10S strip inoculated. A *Shigella* species was identified and confirmed as *Shigella flexneri*.

So far in our study we have only had this one case of *Shigella*. Murphy and Nelson have conducted the largest study done so far and have found that 83% of *Shigella* isolates are *S. flexneri*, and only 10% of cases had noticed recent gastroenteritis symptoms while 47% involved a bloody discharge.

It seems that *Shigella* vulvovaginitis is under-reported worldwide and the infection may occur more frequently than is clinically suspected because routine screening is not done. Our trial is continuing.

### ER: A case study

**Chris Campbell.**

Diagnostic Medlab

This talk followed the case of a 61 year old Samoan male resident in New Zealand since 1961. The patient went to his GP having been unwell for 2 - 3 weeks that started with fever and sweats. In 1994 he had had his mitral and aortic valves replaced. Upon examination by echocardiogram large vegetation around the mitral valve was found. Blood cultures were taken and sent to our Microbiology department. A Gram-positive non-sporing bacillus was isolated from all blood culture bottles at which time the patient was admitted to hospital. This Gram-positive bacillus was identified as *Erysipelothrix rhusiopathiae*. On blood agar *Erysipelothrix rhusiopathiae* forms colonies very similar in appearance to Viridans streptococci. Characteristic biochemical reactions include: H<sub>2</sub>S positive, catalase-negative, and TSI acid slant and butt.

The patient was treated with penicillin G 12MU/day for 6 weeks and the vegetation monitored by echocardiogram. Last examination revealed no visible vegetation and no growth from repeat blood cultures following completion of antimicrobial therapy.

*Erysipelothrix rhusiopathiae* was discovered by Koch in 1876 but it was Loeffler who in 1880 discovered erysipelas in pigs with which it is now commonly associated. This bacterium survives very well in the environment usually entering the host through puncture wounds. Although infections may occur in over 50 different types of animals, pigs are the most commonly infected and can show a variety of symptoms from localised cutaneous infections to endocarditis. Human infection range from localised skin infections to septicaemia and heart disease and is generally found as a result of an occupational hazard. In the case study there was no association with pigs and the only incident that was recorded involved the patient assisting his daughter to gut a fish.

### Cryptosporidium parvum: genetic characterisation and transmission routes of New Zealand isolates

**Jim Learmonth.**

Protozoa Research Unit, Massey University

*Cryptosporidium* is an obligate protozoan parasite of vertebrates causing a self-resolving gastroenteritis in immuno-competent hosts. Of the ten recognized species of *Cryptosporidium*, humans are almost exclusively infected by *C. parvum*. Each mammalian genera tends to be

infected by its own specific *C. parvum* genotype with humans playing host to both the human genotype (genotype 1) and the bovine genotype (genotype 2).

We have examined New Zealand human isolates of *C. parvum* by the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) technique to detect the predominant infecting genotype and to determine transmission routes. New Zealand has two peaks of cryptosporidiosis in most years, coinciding with spring and autumn.

Examination of the *C. parvum* 18S rDNA loci by PCR-RFLP indicated a spring genotype 2 peak (zoonotic transmission route) and an autumn genotype 1 peak (anthroponotic transmission route). Total isolates received from provincial cities were predominantly genotype 2 while the one metropolitan city to send isolates was hosting a *C. parvum* majority of genotype 1. These results presumptively indicate a link between genotype 2 prevalence in provincial centres, spring (calving), and the dairy industry.

#### **Saksenaia vasiformis : a rare cause of zygomycosis**

**Louanne Storey<sup>1</sup>, Arlo Upton<sup>2</sup>, Karen Rogers<sup>3</sup>.**

Microbiology Dept., Middlemore Hospital<sup>1</sup>, Microbiology<sup>2</sup> and Mycology<sup>3</sup> Depts., Auckland Hospital

Zygomycosis is an infection caused by saprophytic fungi of the class *Zygomycosis*, order *Mucorales*. It is usually associated with underlying illness. *Saksenaia vasiformis* is a rare cause of zygomycosis because it

fails to sporulate on commonly used mycology media; laboratory diagnosis can be delayed or not made. Our case highlights the aggressive nature of this infection and in contrast to cases of zygomycosis due to more commonly isolated organisms eg *Rhizopus* species, patients with *S.vasiformis* are less likely to be immunocompromised. The possibility of zygomycosis should not be ignored in a non-immunocompromised patient, particularly if the presentation is cutaneous. A case of cutaneous *S. vasiformis* infection will be presented along with a review of literature involving the organism.

#### **Are you on to IT?**

**Gayleen Boyd, Catherine Stoddart**

Case report.

A 69-year-old man of solid build and average height presented in October 2002 with osteomyelitis of the third lumbar vertebra. He had a history of back pain dating back to 1986. Initial surgery was performed in 1999. His condition again deteriorated in July 2002. Further surgery was performed in December 2002, delayed by underlying diverticular disease. During surgery a swab and tissue were taken for histology and microbiology examination.

After prolonged incubation there was a light growth of *Campylobacter fetus*. This is an uncommon isolate and raised questions concerning incubation times and conditions.

## Haematology

### Special Interest Group

#### HSIG Questionnaire

Journal-British Journal of Haematology- Volume 120, Number 4, February- II 2003, pages 556-573.

Guidelines on the diagnosis and management of the thrombotic microangiopathic haemolytic anaemias

#### Questions

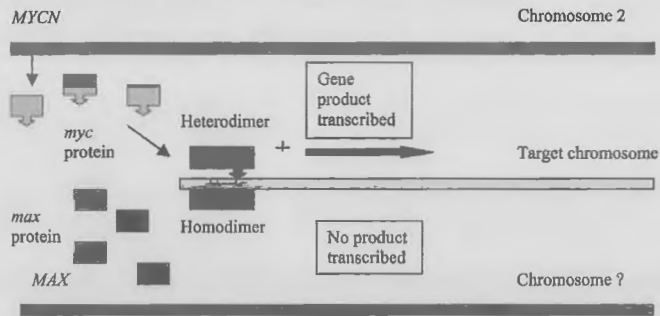
1. State the most common clinical features seen in TTP (Thrombotic Thrombocytopenic Purpura)?
2. State the most common features seen in HUS?
3. What is HELLP Syndrome?
4. What are the blood film findings in TTP?
5. Name 4 precipitants of TTP?

#### True or false

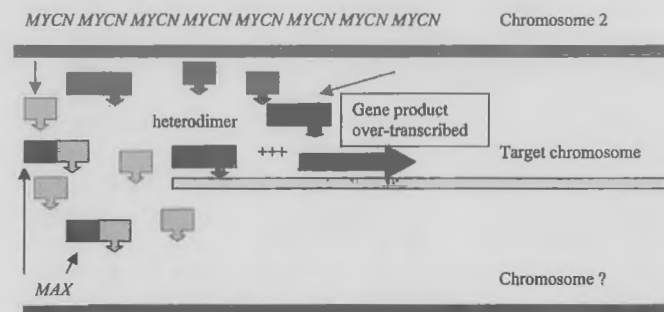
6. The formation of platelet microvascular thrombi is related to the pathogenesis of TTP- True or False
7. Verocytotoxins are implicated in the pathogenesis of HUS- True or False
8. Reduced VWF-Cleaving Protease (VWF-CP) activity is sensitive but not specific to TTP- True or False
9. Prolonged thrombocytopenia for >10 days is associated with long-term renal sequelae in HUS-True or False
10. HELLP occurs in up to 10% of women with severe pre- eclampsia- True or False

Please turn to page 80 for the Answers

**Figure 2a:** MYCN transcription in normal cell nucleus



**Figure 2b.** MYCN transcription in neuroblastoma cell nucleus



## Correction

Mackay RJ. Neuroblastoma: biology of a tumour model. *N Z J Med Lab Sci* 2003; 57 (1): 8-11.

An error occurred in Figure 2 of this article. Figure 2b in this article was printed identical to Figure 2a. Here is the correct Figure 2 for this article.



## Southland Hospital Laboratory Staff Reunion



We are planning a reunion of all past and present Southland Hospital Laboratory employees at Labour Weekend 2004.

If you have worked at the Laboratory please send in your contact details so we can build up a database of all past employees.

Even if you do not intend joining us please reply. This will indicate who has been contacted and who we still have to find.

Please pass this on to any past Laboratory staff, or send us their contact details.

Details and updates will be posted on the Southland Hospital website [www.southlandhealth.co.nz](http://www.southlandhealth.co.nz)

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Email address: \_\_\_\_\_

Address: \_\_\_\_\_ Attending: yes/no \_\_\_\_\_

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PO Box 828,  
Invercargill

<mailto:maree.jackson@sdbh.govt.nz>

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## New products and services

### Thermo Shandon

Earlier this year Thermo Shandon announced the appointment of co distributor Medica Pacifica Ltd to market the Shandon range of Histology, Cytology, Immunochemistry and Mortuary/Autopsy equipment and consumables throughout New Zealand. Thermo Shandon is a well known and established brand in the histology market and the Cytospin is synonymous with Cytology. Two new products recently released by Shandon are the Excelsior Tissue Processor and the Cytospin 4. For further information on these products and orders for Shandon consumables please contact Medica Pacifica Ltd at [info@medica.co.nz](mailto:info@medica.co.nz) or free phone 0800 106 100.

### JRH Biosciences

Medica Pacifica Ltd has been appointed distributor for the JRH Biosciences Cell Culture products. JRH Biosciences manufactures Serum Free Media, Classical Media, Buffers, Supplements, Growth Factors, Protein Components, BioEase Disposable Bags & BioProcessing System. For a complete catalogue contact Medica Pacifica Ltd email [info@medica.co.nz](mailto:info@medica.co.nz) or free phone 0800 106 100

### TPP

TPP Tissue Culture flasks with 'peel off' foil lids now available from Medica Pacifica Ltd. For a complete catalogue phone Medica Pacifica Ltd on free phone 0800 106 100 or email [info@medica.co.nz](mailto:info@medica.co.nz)

### LeptoTek Lateral Flow - an innovative new tool for diagnosing leptospirosis

LeptoTek Lateral Flow is an easy-to-use assay for rapid screening of *Leptospira* specific IgM antibodies in whole blood and human sera. No special equipment or training is needed. Reliable results within ten minutes make it ideal for both laboratory and in-field screening.

The assay is a one-step colloidal gold immunoassay. Broadly reactive *Leptospira-specific* antigen is immobilised as a discrete line on a porous nitrocellulose membrane located in a test zone. The detection reagent consists of mobile red colloidal gold particles labelled with anti-human IgM deposited within the membrane. A positive result will produce a red line in the test zone. A second line in the control zone functions as a reagent control.

Each LeptoTek Lateral Flow (ref 225600) kit includes materials for 25 determinations.

For more information, please talk to your Sales Specialist. You can also contact us by phone (1 800 333 421), fax (1 800 065 421), or email [customer.service@biomerieux.com.au](mailto:customer.service@biomerieux.com.au)

### The first BacT/ALERT 3D Combination Module arrives in Australasia

bioMérieux are pleased to announce that the first BacT/ALERT 3D Combination Module is being installed at Central Queensland Pathology Laboratories in Mackay, Queensland. The BacT/ALERT 3D 120-cell Combination Module integrates blood, body fluid and mycobacteria culture into a single, space saving system. Featuring integrated touch-screen data management with two 60 cell drawers, the BacT/ALERT 3D Combination Module can handle up to 7,200 blood or body fluid cultures or 1,000 mycobacteria cultures annually. The system can be expanded as your needs grow to a maximum capacity of 840 cells. A range of software options are available including full bi-directional interface capability. Like all products in the 3D range, the Combination Module features bioMérieux's proprietary

colourimetric sensor technology, delivering high performance detection with the lowest false positive rate. For more information, please talk to Ian Robertson. You can contact us by phone (0 800 284 825), fax (0 800 284 835), or email ([ian.robertson@as.biomerieux.com](mailto:ian.robertson@as.biomerieux.com)). We publish news and technical information first on [www.biomerieux.co.nz](http://www.biomerieux.co.nz)

### Shatterproof & Lightweight Plastic Bottles are a new milestone in the development of the BacT/ALERT System

We are pleased to announce the development of PLASTIC BOTTLES for use in all BacT/ALERT systems. These new bottles will be introduced after our current stocks of glass bottles are exhausted.

These innovative and patented multi-layer plastic bottles will significantly impact the world of blood culture testing by reducing the risks of accidental injury among laboratory or hospital staff. The plastic used to produce these bottles does not alter the system's detection performance, and furthermore, the plastic bottles will be 30% lighter than the glass bottles currently used on the BacT/ALERT system, and 50% lighter than other commercialised glass blood culture bottles.

The same proven media formulations are used for equivalent recovery to BacT/ALERT media in conventional glass bottles. A new range of safety products for sample collection will also be available. For more information, please talk to Ian Robertson. You can contact us by phone (0 800 284 825), fax (0 800 284 835), or email ([ian.robertson@as.biomerieux.com](mailto:ian.robertson@as.biomerieux.com)). We publish news and technical information first on [www.biomerieux.co.nz](http://www.biomerieux.co.nz)

### Answers to the HSIQ Questionnaire from page 78

1. Thrombocytopenia, microangiopathic haemolytic anaemia, neurological signs, renal impairment and fever
2. Thrombocytopenia, microangiopathic haemolytic anaemia, renal failure
3. Haemolysis, elevated liver enzymes, low platelets
4. Red cell fragmentation and polychromasia
5. Drugs, autoimmune disease, malignancy and infection (*E. Coli*, HIV)
6. True
7. True
8. True
9. True
10. True

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# Abstracts of articles in the *British Journal of Biomedical Science*. Official publication of the British Institute of Biomedical Science

**Ocana VS, Nader-Macias ME. Vaginal lactobacilli: self- and co-aggregating ability. *Br J Biomed Sci* 2002;59:183-90.**

Lactic acid bacteria are the dominant bacteria of the vaginal tract in healthy women. Lactobacillus species form a barrier population that protects from pathogen colonisation by mechanisms that include adhesion to epithelial surfaces, self-aggregation and co-aggregation. In this study, factors involved in the self-aggregating ability of vaginal lactobacilli and in the co-aggregation of these microorganisms with *Candida* spp. are studied. Both self-aggregation and co-aggregation are monitored quantitatively by the decrease in the absorbance of suspensions of the microorganisms and qualitatively by light microscopy. The self-aggregating ability of four vaginal lactobacilli was shown to be caused by a peptide or protein sensitive to trypsin. However, in self-aggregating *Lactobacillus acidophilus* CRL 1294 the factor was resistant to trypsin and sensitive to pepsin. Among self-aggregating lactobacilli, *L. acidophilus* CRL 1294 and *L. salivarius* CRL 1328 were able to co-aggregate with *Candida* spp. The co-aggregating factor for both strains proved to be peptide of the surface and a peptide on the bacterial surface, while the receptor on the yeast was a carbohydrate. Co-aggregation of both lactobacilli and *Candida* spp. was inhibited by the addition of mannose but was not affected by other carbohydrates. Self and co-aggregation factors were not able to induce aggregation in non-aggregating lactobacilli.

**Allam AF, el-Agamy el-SI, Helmy MH. Molecular and immunological characterisation of *Fasciola* species. *Br J Biomed Sci* 2002;59:191-5.**

*Fasciola hepatica* and *F. gigantica* are polymorphic liver flukes that show considerable overlap between species, and various protein separation techniques have been used as alternative means of differentiation. Acid and alkaline polyacrylamide gel electrophoresis (PAGE) show differences between *F. hepatica* and *F. gigantica*. Following SDS-PAGE, *F. hepatica* proteins are characterised by the presence of eight major peptide bands, with molecular weights estimated as 48, 45, 43.5, 37, 33, 29, 27 and 25.5 kDa. In contrast, *F. gigantica* shows only five major protein bands of 57.6, 54, 48, 29 and 27 kDa. Isoelectric focusing (IEF) demonstrates 17 bands from *F. hepatica* and 22 bands from *F. gigantica* between pH 3.5 and pH 10. Although many bands appear common to both species, some are species-specific. Six cases of human acute fascioliasis diagnosed clinically, haematologically and immunologically are also studied. Gel immunodiffusion and immunoelectrophoresis, using adult *F. hepatica* and *F. gigantica* antigens, are used to determine the species, and indicate that the antisera are more specific for *F. hepatica*.

**Orchard G. Evaluation of melanocytic neoplasms: application of a pan-melanoma antibody cocktail. *Br J Biomed Sci* 2002;59:196-202.**

Incidence of malignant melanoma (MM) is rising rapidly throughout the Western world, and the number of melanocytic lesions removed for histological assessment has increased. MM can present with a myriad

of histological appearances that make diagnosis problematic, particularly when dealing with metastatic deposits. Immunohistochemical diagnosis relies on a panel of antibodies comprising polyclonal S100 protein and the monoclonal antibodies HMB 45, MART-1, tyrosinase and, to a lesser extent, NKIC3. Confirmation of problematic cases relies on the use of polyclonal S100 protein, as its sensitivity has yet to be matched by any monoclonal antibody. The introduction of a potentially valuable pan-melanoma cocktail, composed of HMB 45, MART-1 and tyrosinase, is examined in 50 primary cutaneous malignant melanomas, five desmoplastic malignant melanomas (DMM), 35 benign naevi, 20 metastatic malignant melanomas, 10 basal cell carcinomas (BCC) and 10 squamous cell carcinomas (SCC) and compared to individual immunolabelling with S100 protein, HMB 45, MART-1 and tyrosinase. All BCCs and SCCs were negative with all antibodies. S100 protein, MART-1, tyrosinase and the pan-melanoma cocktail were positive for all cases of benign naevi. HMB 45 labelled all junctional and compound naevi, five of the eight intradermal naevi and five of the seven blue naevi. All 50 primary cutaneous MMs were positive with S100 protein, 49/50 with the pan-melanoma cocktail and tyrosinase, 47/50 with MART-1 and 46/50 with HMB 45. Of the five cases of DMM, all were positive with S100 protein and three of the five were positive with HMB 45, MART-1, tyrosinase and the pan-melanoma cocktail. In the case of metastatic MM, all 20 cases were positive with S100 protein, the pan-melanoma cocktail and tyrosinase. MART-1 was positive in 19/20 cases and HMB 45 in 17/20 cases. The pan-melanoma cocktail showed a high sensitivity for all forms of MM and should be considered a complementary marker to polyclonal S100 protein. Results confirmed that currently there is no alternative antibody available to match the sensitivity of polyclonal S100 protein for immunolabelling DMM.

**Womack C, Wade C. How much tissue is present in archival paraffin blocks and slides? How much tissue is present in archival paraffin blocks and slides? *Br J Biomed Sci* 2002;59:203-4.**

In order to inform the debate about tissue blocks and slides introduced by the Retained Organs Commission, a study is undertaken to determine the percentage weight of tissue present in the surgical archive in the cellular pathology department of a district general hospital. When original, unprocessed tissue weight is expressed as a percentage, based on the weight of 100 archival paraffin blocks, values range from 0.2% to 41.5%. When the corresponding archival slides are also included, the values fall as low as 0.1% (i.e. up to 99.9% of the stored archival material for a piece of processed tissue could be non-human material). The results are used to make a case for including archival histological material as part of the patient's clinical record, although it is accepted that this study was performed only on surgical tissue.

**Ogundele MO. Effects of storage on the physicochemical and antibacterial properties of human milk. *Br J Biomed Sci* 2002;59:205-11.**

Storage of human milk for limited periods of time is unavoidable in



neonatal units and also in the home where increasing numbers of mothers go back to work soon after delivery. Many mothers, convinced of the importance of prolonged exclusive or complementary breastfeeding, often express and store human milk for use during the period of separation. This study examines the effects of different storage methods on the pH and some antibacterial activities of human milk. Portions of milk and colostrum samples from healthy lactating mothers were stored at 4 degrees C and -20 degrees C for periods ranging from one day to 12 weeks. The stored milk samples were analysed for pH, bactericidal and bacteria sequestration activities against a serum-sensitive *Escherichia coli*, and compared with freshly collected samples, with and without EDTA. Milk became progressively more acidic during storage. While the bactericidal activities of refrigerated samples diminished rapidly, up to two-thirds of the original activity level was maintained by freezing for up to three months. The ability of milk fat globule membrane to adhere to suspended bacteria was gradually lost in frozen milk samples, while it was greatly enhanced during the first few days in refrigerated samples, before declining sharply. This study shows that loss of bactericidal activity in refrigerated milk is well compensated for by enhanced bacteria sequestration activity, and allays any fears that might arise concerning the suitability of stored human milk for infant consumption.

**Osman MY, Sharaf IA, el-Rehim WM, el-Sharkawi AM. Synthetic organic hard capsule colouring agents: in vitro effect on human true and pseudo-cholinesterases. *Br J Biomed Sci* 2002;59:212-7.**

Hard capsules are made of pure gelatin and small quantities of additives, including colouring agents permitted for use in food. In this study, the effects of three colouring agents (sunset yellow, quinoline yellow and erythrosine) on true and pseudo-cholinesterases (ChE) are assessed in erythrocytes and plasma, respectively. Results indicated that the synthetic compounds affected both true and pseudo ChE activity. The concentration of sunset yellow which caused 50% inhibition (IC<sub>50</sub>) of true ChE was about 64% that of pseudo-ChE; for erythrosine, IC<sub>50</sub> was approximately the same for both true and pseudo-ChE; and for quinoline yellow, IC<sub>50</sub> for true ChE was 25% of pseudo-ChE, although its effect on both true and pseudo-ChE was greater than seen with the other two dyes. Inhibitions of both true and pseudo-ChE were of mixed type (competitive and non-competitive). The enzyme-inhibitor dissociation constant (K<sub>i</sub>) indicated that quinoline yellow was most potent and erythrosine was least potent out of the three compounds. Inhibition of both true and pseudo-ChE by each of the three dyes was abolished by dialysis, indicating that the effects were reversible.

**Sreenivas G, Ansari NA, Singh R, Subba Raju BV, Bhatheja R, Negi NS, Salotra R. Diagnosis of visceral leishmaniasis: comparative potential of amastigote antigen, recombinant antigen and PCR. *Br J Biomed Sci* 2002;59:218-22.**

Development of simple, economical and non-invasive tests for the early diagnosis of visceral leishmaniasis (VL) or kala-azar (KA) remains a challenge, and serological studies based on antigen prepared from the amastigote stage of *Leishmania donovani*, the stage that causes infection, are lacking. In the present study, circulating antibodies to total antigen isolated from the promastigote and amastigote stages of the parasite, as well as to recombinant K39 (rK39) antigen, are measured by enzyme-linked immunosorbent assay (ELISA) and the results compared with a polymerase chain reaction (PCR) test for KA diagnosis. In 116 samples of KA examined, the amastigote antigen gave significantly higher mean absorbance values in ELISA than did the

promastigote antigen. The sensitivity for KA detection was significantly higher using the amastigote antigen (94%) than the promastigote antigen (90.5%). Analysis in 91 controls showed that specificity was higher with amastigote antigen (92.3%) than with promastigote antigen (86.8-89.0%). Reliability of ELISA diagnosis with amastigote antigen was only marginally lower than that with rK39 ELISA or with the PCR test. Easy availability and low cost of indigenous amastigote antigen, together with the simplicity of ELISA compared with PCR, make ELISA based on amastigote antigen a promising choice for the diagnosis of KA.

**Spence N. Electrical impedance measurement as an endpoint detection method for routine coagulation tests. *Br J Biomed Sci* 2002;59:223-7.**

A whole-blood platelet aggregometer is adapted to measure electrical impedance changes in plasma during prothrombin time (PT) estimations. The impedance curve shows an acceleration phase, which is comparable to the absorbance curve acceleration phase associated with the onset of coagulation. The amplitude of the impedance change correlates with the fibrinogen concentration of the plasma. Statistical analysis of PT derived by absorbance and impedance changes shows a significant difference between the two methods but a good correlation. The method is reproducible but laborious and requires attention to technique. Further investigation of the method utilising a more sensitive instrument and redesigned electrodes is indicated. It may also be possible to modify reagent systems to optimise impedance changes.

**Moody AH, Chiodini PL. Non-microscopic method for malaria diagnosis using OptiMAL IT, a second-generation dipstick for malaria pLDH antigen detection. *Br J Biomed Sci* 2002;59:228-31.**

Rapid diagnostic tests for malaria are now a commonly used procedure for malaria diagnosis. New or improved devices need to be evaluated against a recognised gold-standard procedure and subjected to conditions of temperature and humidity that may affect their performance. The OptiMAL 48 RDT has now been available commercially for several years and a second-generation OptiMAL IT test is now coming onto the market. In this study the problems associated with the routine use of OptiMAL 48 is investigated and its performance compared with a second-generation individual test, OptiMAL IT. Sensitivity and specificity for detection of all malaria species for both tests were comparable but loss of sensitivity of the test strips due to humidity or temperature found with the routine use of OptiMAL 48 was not seen with the individual OptiMAL IT. False-positive results for *Plasmodium falciparum*, seen in two negative blood samples, were attributed to the presence of high levels of heterophile antibodies.

**Clarke SC. Bioterrorism: an overview. *Br J Biomed Sci* 2002;59:232-4.**

Bioterrorism has reached the forefront of the public imagination following recent events across the world. The disaster of 11 September 2001, followed by anthrax letters sent via the US postal system and now renewed tension over Iraq have all brought the possibility of bioterrorism closer. A number of biological agents could be used in a terrorist attack, including anthrax, plague, smallpox and botulinum toxin. The serious diseases that these agents produce have been brought under control in the developed world; however, a lack of protective immunity against such diseases could cause considerable

morbidity and mortality if used in a terrorist attack. This essay provides a background to bioterrorism, discusses many of the current points of interest and gives an update to the economic consequences of such an attack.

**Jewell AP. Role of apoptosis in the pathogenesis of B-cell chronic lymphocytic leukaemia. *Br J Biomed Sci* 2002;59:235-8.**

B-cell chronic lymphocytic leukaemia (B-CLL) is a clinically heterogeneous disease characterised by the accumulation of a clonal population of B lymphocytes. This accumulation is considered to result from the prolonged survival of B-CLL cells arrested in the G0 stage of the cell cycle. However, when cultured in vitro, B-CLL cells die rapidly by apoptosis. It is now clear that a number of factors can delay or postpone the onset of apoptosis, including a number of cytokines and direct contact with different cell types. Although many drugs are now known to cause clinical improvement in B-CLL by causing apoptosis of B-CLL cells, in only a few cases have biological mechanisms been reported to have similar effects. It is now important to understand the role of these mechanisms in the pathogenesis and progression of B-CLL, and to devise strategies to exploit them for therapeutic use.

**Al-Jabri AA, Nzeako B, Al Mahrooqi Z, Al Naqdy A, Nsanze H. In vitro antibacterial activity of Omani and African honey. *Br J Biomed Sci* 2003;60:1-4.**

The study aims to investigate the antibacterial activity of honey obtained from different parts of Oman and compare it with that of honey obtained from elsewhere in Africa. A total of 24 honey samples (16 from different parts of Oman and eight from elsewhere in Africa) were investigated for their antibacterial activity against *Staphylococcus aureus* (NCTC 6571), *Escherichia coli* (NCTC 10418) and *Pseudomonas aeruginosa* (NCTC 10662) using standard antimicrobial assays. Marked variations in the antibacterial activity of the different honey samples were observed. Fourteen of the 16 Omani samples and five of the eight African samples showed antibacterial activity ranked as either fair, good or excellent to at least one of the three bacterial strains tested. Both Omani and African honeys possess in vitro antibacterial activity against the three bacterial strains tested, with 25% of the samples showing excellent antibacterial activity.

**Brockes C, Buchli C, Locher R, Koch J, Vetter W. Vitamin E prevents extensive lipid peroxidation in patients with hypertension. *Br J Biomed Sci* 2003;60:5-8.**

Oxidative modification of low-density lipoprotein (LDL) increases atherogenic potential to induce the accumulation of lipids and cells in the vascular wall. Previous studies reveal that hypertensive patients have a higher susceptibility to LDL oxidation. As animal models indicate that vitamin E protects LDL from oxidation, here we study the influence of vitamin E on the resistance of LDL to oxidation (lag time) in 47 subjects (31 normotensive, 16 hypertensive) before and after oral administration of vitamin E (400 IE) daily for two months. LDL was isolated and oxidised by incubation with copper ions. The time course of oxidation was measured by continuous photometric monitoring of diene formation at 234 nm. At the beginning of this study, normotensive subjects showed a lag time of 108 +/- 26 minutes and hypertensive patients a lag time of 85 +/- 24 minutes ( $P < 0.05$ ). Vitamin E caused a significant increase in the lag time in both groups: normotensive subjects 128 +/- 33, hypertensive patients 114 +/- 27 minutes ( $P < 0.01$ ). At completion of the study, lag times in both groups were similar ( $P = \text{not significant}$ ). The data presented here suggests that

vitamin E protects against the increased risk of vascular disease in patients with hypertension by reducing the susceptibility to oxidative modification of LDL. Vitamin E may therefore act as an inhibitor of atherogenesis.

**Chen YN, Chen SY, Zeng LJ, Ran JM, Xie B, Wu MY, Wu YZ. Secondary sulphonylurea failure: what pathogenesis is responsible? *Br J Biomed Sci* 2003;60:9-13.**

Sulphonylurea (SU) stimulates insulin secretion by pancreatic beta-cells and is generally used as a first-line treatment for type 2 diabetes. However, after long-term SU treatment (six months or over), some patients begin to show an increase in blood glucose once again (secondary SU failure). Two theories have been put forward to explain this failure—dysfunction of the proinsulin conversion machinery or insulin resistance. However, the primary pathogenesis behind secondary SU failure still needs to be investigated. Using a reliable technique that specifically identifies intact proinsulin (IPI), total proinsulin (TPI) and specific insulin (SI), this study aims to discover if a defect in the proinsulin converting mechanism plays a role in SU failure. Three groups were recruited for this study: healthy controls ( $n=8$ ), SU responders ( $n=38$ ) and secondary SU failures ( $n=46$ ). Serum concentrations of insulin-related molecules released in response to a standard glucose challenge test were compared between the groups. It was found that total SI was lower in the patient groups ( $P < 0.05$  compared to the control group), while TPI and IPI showed no distinct difference between the three groups ( $P > 0.05$ ). TPI:SI ratio and IPI:SI ratio showed marked increases in the patient groups ( $P < 0.05$  compared to control group), with no obvious quantitative difference between SU responders and secondary SU failures ( $P > 0.05$ ). Similar results for the Homa Insulin Resistant Index were found between the two patient groups. Interestingly, blood glucose at 180 mins after glucose challenge was significantly higher in the secondary SU failure group ( $P < 0.05$ ), with no correlation to SI, while the SU responder group showed good correlation between the parameters ( $P < 0.05$ ). We conclude that type 2 diabetes is associated with obvious dysfunction in the proinsulin-converting process and shows severe SI deficiency in responding to glucose challenge. Dysfunction of the proinsulin conversion mechanism was not an extra cause responsible for SU failure.

**Feighery L, Collins C, Feighery C, Mahmud N, Coughlan G, Willoughby R, Jackson J. Anti-transglutaminase antibodies and the serological diagnosis of coeliac disease. *Br J Biomed Sci* 2003;60:14-8.**

Tissue transglutaminase (tTG) has recently been identified as the antigenic target recognised by anti-endomysial antibodies in patients with coeliac disease. In this study, an enzyme-linked immunosorbent assay (ELISA) is used to measure IgA, IgG and IgM antibodies to tTG in patients with coeliac disease and a variety of other inflammatory disorders; and is compared to the standard immunofluorescence test used to detect endomysial antibodies (EMA). In the samples tested, 3% control sera ( $n=146$ ), 83% EMA-positive sera ( $n=29$ ), 9% patients with Graves' disease ( $n=94$ ), 12% antimitochondrial antibody-positive sera ( $n=53$ ), 11% rheumatoid arthritis patients ( $n=53$ ) and 22% systemic lupus erythematosus (SLE) patients ( $n=46$ ) were positive for anti-tTG antibodies. In contrast, none of the controls, 1% of patients with Graves' disease, 2% antimitochondrial antibody-positive sera, 2% rheumatoid arthritis patients and none of the SLE patients were positive for EMA. Measurement of IgG or IgM antibodies to tTG was much less reliable than IgA anti-tTG antibody for the serological

diagnosis of coeliac disease. The addition of calcium to the coating buffer improved the assay characteristics of the anti-tTG ELISA. However, the IgA anti-tTG ELISA, with and without calcium, performed less well than the standard EMA test used for the serological diagnosis of coeliac disease. In particular, the anti-tTG ELISA gave a higher rate of non-specific positive reactions.

**Mettimano M, Specchia ML, Ianni A, Arzani D, Ricciardi G, Savi L, Romano-Spica V. CCR5 and CCR2 gene polymorphisms in hypertensive patients. *Br J Biomed Sci* 2003;60:19-21.**

Essential hypertension is a complex trait under polygenic control. Evidence suggests immune system involvement during pathogenesis. CC-chemokine receptor (CCR)5 and CCR2 are characterised by gene polymorphism. Variant alleles are derived from a deletion in the CCR5 gene (CCR5delta32) and a substitution mutation at the CCR2 locus (CCR264I). CCR polymorphic forms have been studied extensively as invasion cofactors for HIV-1, but they have also been implicated in immuno-related disorders. Here, we evaluate the allelic distribution of CCR5 and CCR2 genes in essential hypertension in a case-control study. Genotype frequency in a group of essential hypertensive patients (stage I-II; n=120) and a group of unrelated, healthy Caucasian subjects (n=340) is compared. CCR gene polymorphism is analysed by polymerase chain reaction and restriction enzyme digestion. A statistically significant difference was observed for CCR5 and CCR2 mutant alleles in essential hypertensive patients, compared with the controls (P=0.004 and P=0.003, respectively). CCR5delta32 and CCR264I alleles showed a 0.096 and 0.10 frequency among cases. To date, a role for the immune system in hypertension has not been clarified, nor has the predictive value of CCR polymorphisms.

**Liu CH, Ma WL, Shi R, Zhang B, Ou YQ, Zheng WL. Gene expression study of *Saccharomyces cerevisiae* with the Agilent 2100 bioanalyser. *Br J Biomed Sci* 2003;60:22-5.**

This study explores the restriction display-polymerase chain reaction (RD-PCR) application of a new chip-based nucleic acid analysis system (Agilent 2100 bioanalyser) in a gene differential expression study. Total RNAs are extracted from *Saccharomyces cerevisiae*, double-stranded complementary DNA (cDNA) is synthesised by reverse transcription from the purified messenger RNA (mRNA), RD-PCR conducted to obtain the cDNA fragments and bioanalyser and agarose gel electrophoresis compared for the analysis of RD-PCR products. The bioanalyser proved to be faster and more sensitive in separating and detecting gene fragments, and was also able to compare different gene fragments quantitatively. Using this technology, comparison of several differential gene fragments is performed.

**Jennings I, Cooper P. Screening for thrombophilia: a laboratory perspective. *Br J Biomed Sci* 2003;60:39-51.**

The worldwide annual incidence of venous thrombosis is estimated at 1 in 1000 individuals, and associated pulmonary embolism represents a major cause of morbidity and mortality. Thrombophilia may be an inherited or acquired condition, with the former identified in approximately 25-30% of patients with thromboembolic disease. Recently published guidelines on thrombophilia testing recommend assays for protein C, protein S and antithrombin; a modified activated protein C resistance test (with factor V-deficient plasma); polymerase chain reaction for prothrombin G20201A, together with prothrombin time, activated partial thromboplastin time, thrombin clotting time and assays to detect antiphospholipid antibodies. This review highlights

some of the issues that laboratories should consider when employing tests for the diagnosis of thrombophilia.

## Other articles without abstracts

Murayama O, Tsuboi Y, Samata T, Moore JE, Matsuda M. Biochemical isolation and identification of DnaK and GroEL from urease-positive thermophilic campylobacters. *Br J Biomed Sci* 2003;60:26-7.

Moore JE, Wareing DR. Phenotypic diversity of campylobacter isolates from sporadic cases of human enteritis in Northern Ireland. *Br J Biomed Sci* 2003;60:28-30.

Moore JE, Stanley T, Canney A, Russell L, Matsuda M, Murphy PG. Multilocus enzyme electrophoresis typing of clinical *Helicobacter pylori*. *Br J Biomed Sci* 2003;60:30-4.

Clarke L, Millar BC, Moore JE. Extraction of genomic DNA from *Pseudomonas aeruginosa*: a comparison of three methods. *Br J Biomed Sci* 2003;60:34-5.

Conroy S, Wassif WS. Hyperparathyroidism—pitfalls in management. *Br J Biomed Sci* 2003;60:35-6.

Tamimi W, Abokhashab M, Alhazmi Z, Alsadhan A, Al-Mutair A, Al-Alwan I, Al-Saif S, Hajeer AH. Congenital hypothyroidism in Saudi children. *Br J Biomed Sci* 2003;60:37-8.

# Abstracts of articles in the *Australian Journal of Medical Science*. Official publication of the Australian Institute of Medical Science

***Vibrio mimicus* septicaemia associated with acute cholecystitis: a case report. Haswell HM, Forgan-Smith R. *Austr J Med Sci* 2003;24(1):4-9**

Septicaemia caused by *Vibrio mimicus* is reported in a patient with acute cholecystitis. *V. mimicus* is closely related to *Vibrio cholerae* type non-O1 and causes similar clinical symptoms including diarrhoea and gastroenteritis. This organism has also been reported from ear and wound infections. It has rarely been reported from blood, this being the third published case. Technical aspects of isolation and identification are described.

**Head lice and body lice: shared traits invalidate assumptions about evolutionary and medical distinctions. Bailey AM, Procriv P, Petersen HP. *Austr J Med Sci* 2003;24(1):48-62.**

Human louse-borne diseases are reemerging overseas and biological study of their arthropod vector, *Pediculus*, is needed. In an observational study on head lice, morphological features on the surface of the human host and on closely associated personal objects, such as clothing, influenced location of lice. Given optimal conditions, head lice readily transferred to the body and clothing, fed on the 'naked' body, retreated into, and laid eggs in, clothing or body hair, from which viable nymphs hatched and in turn continued the life cycle. Both wild

and colonised head lice developed and reproduced without scalp contact or hairs and withstood periodic separation from the host. Further, head lice elicited the same dermatopathological effects as are reported for body lice. The belief that only established body lice infestations are a public health threat is challenged; an alternative disease process is proposed; and further basic research into this parasite is strongly indicated.

**The professional status of medical science in Australia. McGregor L, Moriarty H. *Austr J Med Sci* 2003;24(1):63-78.**

Medical science in Australia has encountered barriers to professionalisation that are globally common to medical science and other health occupations. This paper explores what factors determine professionalisation. It also examines some of the processes which health workers have followed in order to obtain the title of "professional". The relatively unsuccessful professionalisation of Australian medical science is explored and factors which may have influenced this are discussed. Some of these issues include a low public profile, a lack of public respect for the work undertaken by the medical scientist, a lack of unity within the group, a lack of autonomy, low income, reduced employment opportunities and restricted continuing education and research opportunities.

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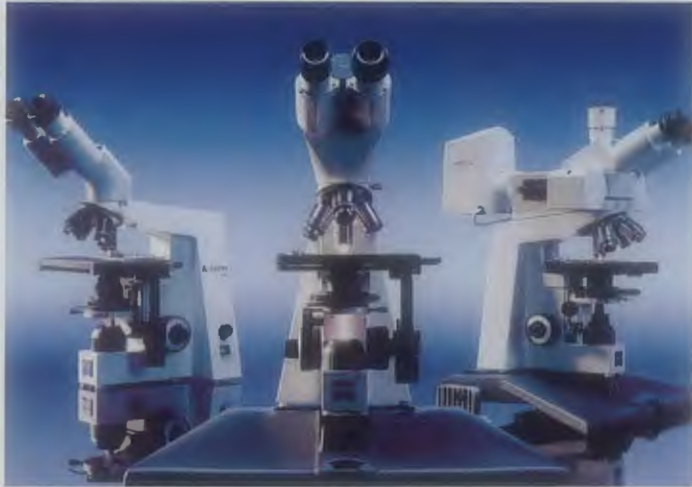
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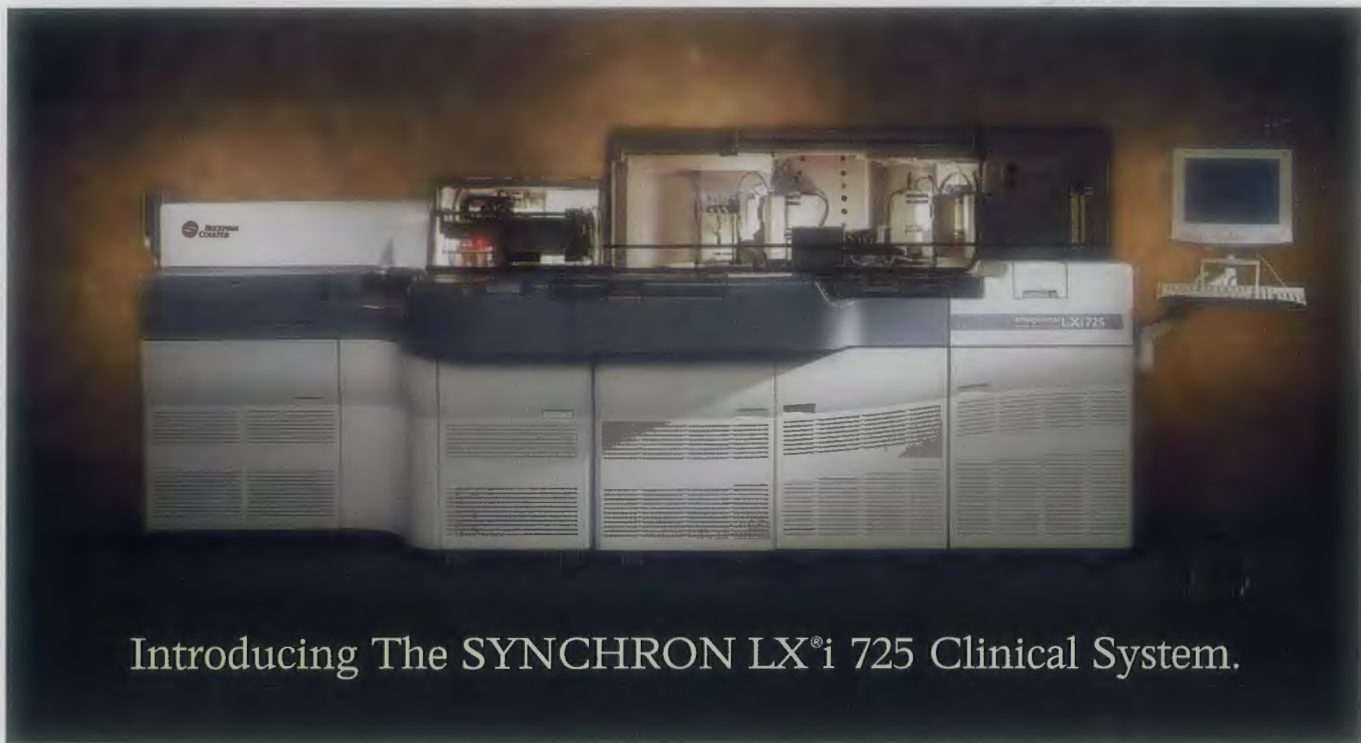


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Thinking about the difference an LXi 725 can make? There's never been a better time to visit us at [www.beckmancoulter.com/lxi725](http://www.beckmancoulter.com/lxi725), or to call your Beckman Coulter representative.



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