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## Editorial



## Review Articles



## Historial Articles



## Book Reviews



## Report



## Regular features

# Editorial

## The marriage between the Institute and the Journal - a diamond celebration

This year we will celebrate the 60th year of publication of the New Zealand Journal of Medical Laboratory Science and as such it is important to look back and see how far we have come. In 1946 the Journal was seen as vital for the distribution of information and sharing of knowledge among the profession in New Zealand. The same is true in the 21st century.

During the Journal's 60 years eleven Editors or Co-Editors have contributed a great deal of their time and energy into developing each issue. Each Editor, in turn, has assembled an Editorial Committee accordingly. Changes under these Editors have often been significant and endured the test of time. The microscope logo, for example, first appeared on the cover of the Journal in 1957. It has remained as a symbol of the profession ever since.

Advertising has always played a significant part in the publication of the Journal. The first issue did not contain any advertising, but from the second issue forward, advertising has become integral. In 1983 colour advertisements were published in the Journal for the first time, and as recognition of the significance of advertising an Advertising Manager, Trish Reilly, was appointed in 1983, a vital role she continues to this day.

Regular features have become an important component in the make up of the Journal. The Pacific Way column has been a featured article since 1983. Continuing education has made an impact since 1984 and the recent introduction of the CPD programme should hopefully have a significant impact on the content of the Journal in the near future.

Today the Journal is distributed widely and abstracted by many overseas services. Contributions from the profession have always been, and will continue to be, vital to the success of the Journal. The past 60 years has seen many interesting and varied articles that have enriched and entertained us. Let's hope this continues for another 60 years and beyond.

To celebrate our diamond anniversary a special Journal prize is being offered for the best case study accepted and published in the Journal during 2006. Despite this prize having been brought to the attention of the Institute's members since August 2005, we have to date not received any submissions. Time is running out as it can take up to two to three months from submission for the article potentially being accepted for publication in the Journal due to the peer review process and subsequent revisions. Having attended many SIG meetings and others, we are aware that there have been many interesting case studies presented at these meetings. Why not submit these to the Journal and inform and teach your colleagues who may not have heard your presentation. Also, you could win this special prize worth $500 and also be eligible to win the Med-Bio Journal award for the best article in each issue of the Journal.

The Journal started its life in 1946 as the *Journal of the New Zealand Association of Bacteriologists*, changing its name to the *New Zealand Journal of Medical Laboratory Technology* in 1960, and to its current title, the *New Zealand Journal of Medical Laboratory Science* in 1990. In this issue of the Journal, and in subsequent issues during 2006, we will reproduce some of the historical papers, editorials, letters, and Council news items published during the formative years of the Journal. In this issue you will see an original article by G. McKinley, a previous Council Member, Secretary, Vice-President, and President from 1945 to 1957, on the laboratory diagnosis of tuberculosis from the January 1947 issue. Also, read the very first Editorial from the first Editor, D. Whillans, and a Letter to the Editor from the first President, E. Buxton. In the latter, he urges the Society to actively pursue the question of registration.

A new feature from this issue on will be a Journal-based questionnaire. There will be 10 questions relating to all material in the Journal, in the format of true/false. You will have to get a minimum of 7 right answers to earn 5 CPD points. Post or fax your answers within four weeks to the NZIMLS office and you will subsequently be notified after the Editor and Deputy-Editor have marked your submitted answers. The questions can relate to any material in the Journal, so read it from cover to cover in order to be able to answer the questions.

No doubt further changes to the Journal will be made over the years to come. However, one fundamental point will not change. That is, the Journal always has, and will continue to publish quality peer-reviewed articles of interest to the profession. In order to do so, the Journal is dependent on the profession to submit their work. Writing scientific articles is not easy and takes a lot of time and effort. The Journal's Editor, Deputy-Editor and Editorial Board Members are "user friendly" and are always willing to help potential contributors. Also, we run how to write articles for the Journal workshops at the Annual Scientific Meetings and will again do so for this year's meeting in Napier. Expect our Editorial team to tap you on the shoulder should you be presenting work at various upcoming scientific meetings. In order to survive, the Journal needs your input. We look forward to your help for the next 60 years.

***Ann Thornton, FNZIMLS, Deputy-Editor***
***Rob Siebers, FNZIMLS, Editor***
***Wellington School of Medicine & Health Sciences,***
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# Myelofibrosis with myeloid metaplasia

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## Abstract

Myelofibrosis with myeloid metaplasia (MMM) is a member of the chronic myeloproliferative disorder group as defined by the World Health Organisation. The disease is uncommon and is initially characterised by anaemia and cytopenia caused by the over-expression of growth factors released by malignant bone marrow megakaryocytes. Increased levels of several growth factors stimulate the production of collagenous proteins by bone marrow fibroblasts. Over time a leucoerythroblastic blood picture and teardrop poikilocytes are seen in the peripheral blood smear and progression to acute leukaemia is seen in some cases. MMM is difficult to treat, however, research into the mechanisms of fibroblast stimulation has led to a number of potentially useful drugs that may progress to clinical trials. Currently the treatment of MMM is palliative in most, with bone marrow transplant the only real curative option for selected patients.

**Key words:** myelofibrosis, metaplasis, leucoerythroblastic, fibrosis, PDGF, tear-drop poikilocytes, TGF-β1, basic fibroblast growth factor

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## Introduction

Myelofibrosis with myeloid metaplasia (MMM) is a form of chronic idiopathic myelofibrosis (CIMF) and is classified as a chronic myeloproliferative disorder (MPD) (1). MMM is a neoplastic clonal haematopoietic stem cell disorder characterised by an intense bone marrow stromal reaction leading to collagen fibrosis, osteosclerosis, and angiogenesis (2-4). Progressive fibrosis of the bone marrow leads to the development of anaemia and hepato-splenic as well as non-hepato-splenic extramedullary haemopoiesis with marked hepatosplenomegaly (3-5). The blood film shows an elevated number of peripheral blood haematopoietic precursors (mean levels 8 to 167 fold higher) and ineffective haematopoiesis that produces varying degrees of cytopenia. In the bone marrow there is evidence of a dysplastic-megakaryocytic hyperplasia (6-9).

## Classification

MMM is classified as chronic idiopathic myelofibrosis (CIMF) under the World Health Organisation's classification of myeloid neoplasms (1). Other members of the group include chronic myelogenous leukaemia Ph+ (CML), chronic neutrophilic leukaemia, chronic eosinophilic leukaemia/hypereosinophilic syndrome, essential thrombocythaemia (ET), and polycythaemia vera (PV) (1).

Among the chronic myeloproliferative diseases, MMM is the least prevalent with an incidence rate that varies between 0.3 to 1.5 per 100,000 people (6). MMM is one of the most difficult diseases of the group to manage and has an average age at diagnosis of approx. 60, which includes some examples seen in the young (4). The average life expectancy following diagnosis is estimated to be three to five years, but may approach 15 years in young patients with good prognostic features (3,5). The disease affects quality of life for the patient, requiring red blood cell transfusions and the markedly enlarged spleen is often accompanied by mechanical discomfort and profound constitutional symptoms (6). Eventually, progressive haematopoietic failure and/or transformation to acute leukaemia leads to the patients' demise (7).

## Genetics

The genetic cause of the MPD is unknown but is thought to arise from a somatic cell mutation in a pluripotent haematopoietic stem cell affecting the myeloid lineage (9,10). During the course of the disease additional clonal alterations are often acquired which are thought to be linked to the progression of chronic MPD into acute leukaemia (11). The genetic mutation(s) in the stem cell that produces MMM remains elusive with about 30% of patients with MMM or PV showing evidence of clonal karyotypic abnormalities at diagnosis (5,6). In these cases there is no common defect providing a link to the genetic abnormality that produces MMM (5,12).

Among the gene defects that have been reported, the most common chromosomal abnormalities are del(13)(q12q14), del (20)(q11q13), partial trisomy 1q, trisomy 8, and abnormalities in chromosomes 1, 7, 9, (13). These are also findings common among other examples of haematologic malignancies (14,15). More recently Dingli and colleagues described an association between a rare chromosomal breakpoint abnormality der(6)t(1;6)(q21-23;p21.3) and MMM, in three out of 81 cases studied (16).

## Aetiology and pathogenesis

MMM can present as a sporadic disease or develop in the setting of either PV or ET after 15-20 years. MMM is unique among the MPD group in displaying the intense bone marrow stromal reactions of collagen fibrosis, osteosclerosis, and angiogenesis (8,17). The disease is characterised by polyclonal fibroblast proliferation and alterations in both cellular and extracellular levels of various fibrogenic and angiogenic cytokines (5). At present it is thought that the stromal reaction is a process mediated by cytokines produced by BM cells that have undergone neoplastic clonal proliferation (6). In MMM both megakaryocytes and monocytes exhibit hypersensitivity to thrombopoietin and have been implicated as the sources of the cytokines that amplify fibroblast proliferation (3,4,11).

Patients with MMM have been shown to have markedly increased levels of fibrosing growth factors. The major megakaryocyte-derived cytokine implicated is TGF-β1 which may interact with thrombopoietin, the major growth factor for megakaryocyte development, to stimulate fibroblasts (6,11,16). Other growth factors and cellular proteins have been shown to contribute to the fibrotic reaction. These include platelet-derived-growth factor (PDGF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF, FGF-2) and calmodulin (3,5,18).

Fibrosis of the BM is the prominent histological feature of MMM with the early stages characterised by the formation of delicate reticulin fibres followed by the development of trichrome-positive fibres and finally obliterative collagenisation. In advanced cases osteosclerosis can also be seen (16). Gradual progression of the BM fibrosis leads to the involvement of other organs with extra-medullary haemopoiesis in the liver and spleen. Hepato/splenomegaly leads to abdominal distension, discomfort, gastrointestinal dysfunction and severe pain in some patients (5,8).

## Laboratory diagnosis

MMM is a disease that can present with a mixed blood picture. The findings of a leucoerythroblastic blood picture a condition characterised by the presence of leucoerythroblasts (granulocyte precursors and nucleated red cells) and poikilocytes (6,8), together with organomegaly and myelofibrosis of the bone marrow, is strongly suggestive of MMM (17). Diagnosis is based on the exclusion of other clonal or non-clonal disease processes that can also be associated with bone marrow fibrosis (2,6). A leucoerythroblastic blood picture is not entirely diagnostic of MMM as other disorders that can produce a similar blood picture include CML, myelodysplastic syndromes (other than MMMl), metastatic cancer, granulomatous infection (5), lymphoma, Hodgkin's disease, and plasma-cell dyscrasias (9).

The blood film morphology at presentation varies depending on whether the patient is first observed during the prefibrotic or the fibrotic stages of the disease. During the early stages, when the marrow is hypercellular, there is often thrombocytosis and sometimes leucocytosis rather than cytopenias (9,10). In addition, the leucoerythroblastic blood picture and poikilocytes may be absent but as the disease progresses, leucopenia, neutropenia, and thrombocytopenia are present and a leucoerythroblastic blood film with teardrop poikilocytes is usually seen. (9). The underlying cause of the leucoerythroblastosis and the production of poikilocytes is intramedullary sinusoidal haematopoiesis in the bone marrow and hepato-splenic, as well as non-hepato-splenic extramedullary haemopoiesis (5,6).

In the final phase of myelofibrosis, a progressive rise of the WBC can be seen with counts of 100-200 x $10^9$/l possible (7). At this time the blood film shows increasing numbers of blasts, promyelocytes and myelocytes. Eosinophilia and basophilia may also be seen in this phase and the blood film may be indistinguishable from that of CML. In other patients an acute transformation with a rapid rise in the blast count, a worsening anaemia, neutropenia, and thrombocytopenia can be seen (2,5).

Examination of the bone marrow is essential in the diagnosis of MMM to establish cause myelophthisis and bone marrow fibrosis (8). A careful morphologic examination is necessary to rule out MDS with myelofibrosis, acute myelofibrosis and fibrosis caused by metastatic cancer (6). In patients with MMM the bone marrow is not easily aspirated, often resulting in a "dry tap" (6). In addition, the result of aspiration alone, if successful, is not diagnostic, with trephine biopsy required to demonstrate BM fibrosis. The most common findings in the core biopsy specimen are medullary fibrosis, neutrophilic and megakaryocytic hyperplasia, and osteosclerosis (6,10). The dysplastic megakaryocytes are often abnormal in morphology with "cloud-like" immature nuclei, with both micro- and macro-megakaryocytes present (10,12). Granulocytes may show hyper-lobulation and erythroid precursor numbers may be normal or increased (9).

Cytogenetic studies of the bone marrow are useful to exclude Philadelphia chromosome positive CML from the other members of the MDS (6). This is of diagnostic importance, particularly if blasts are also present in the blood (2). BM biopsy may also be helpful in the evaluation of other characteristic but non-specific abnormalities that complement the morphological distinction between MMM and related fibrotic disorders (6).

Clonal cytogenetic abnormalities occur in approximately 50% of patients with MMM, but few are useful in the diagnosis (8). Most commonly deletions at 13q-, 20q-; monosomy or long arm deletions of chromosome 7, and trisomy 1q, together with +8 and/or +9 have been reported (5,13). A new chromosomal breakpoint abnormality der(6)t(1;6)(q21-23;p21.3) has recently been reported to have association with MMM (16).

## Prognosis

Among the chronic MPD group, MMM has the worst prognosis with a median survival time of 3.5 to 5.5 years (6,9). There is considerable variation in survival based on the presence or absence of defined prognostic variables (8). In almost all the studies of MMM, advanced age and anaemia correlated with a short-term survival (6,8). Additional negative prognostic indicators include the presence of hypercatabolic symptoms (weight loss, profound fatigue, night sweats, low-grade fever), leucocytosis, leucopenia, peripheral blood blasts, increased numbers of granulocyte precursors, thrombocytopenia, and cytogenetic abnormalities (2,8).

On the basis of combinations of these variables a prognostic model or scoring system has been developed to aid in decision-making about therapy (6). Preferred for its simplicity and reliability, the LILLE scoring system allows for the classification of patients into 3 distinct groups based on haemoglobin and total white cell count (WBC) at diagnosis. The system categorises patients into low-risk (Hb >100g/l and a WBC > 4.0 and <30.0 x $10^9$/l), and high-risk (Hb <100g/l with either WBC < 4.0 and > 30.0 x $10^9$/l), and intermediate, based on these values (9,19).

Median survival may exceed 10 years in the low-risk group, but may be less than two years in the high-risk group (8). Two biological features have recently challenged the traditional prognostic classifications. These are the extent of bone marrow neoangiogenesis, and the number of circulating progenitor cells (2,9). Survival has been shown to be significantly shorter in patients with a grade 3 or 4 increase in angiogenesis (median survival 155 months versus 58.6 months) (6). Patients who have elevated progenitor (CD34+) cell levels have a blast transformation rate in approximately 40% of cases, as compared to 3.4% of patients with a low CD34+ count (2). In one study of MMM patients, blast transformation was seen within 11 months from the date of evaluation in 50% of patients with a high CD34+ cell count (9,20).

The prognostic value of hepatomegaly, splenomegaly, and gender is uncertain. Similarly, the histological features of the bone marrow, including the degree of fibrosis or osteosclerosis, may be of limited value (3,6). The major causes of death in patients with MMM are infection, thrombo-haemorrhagic events, heart failure, and leukaemic transformation. Leukaemic transformation occurs in approximately 20 percent of patients during the first ten years of the disease (6).

## Treatment

Conventional drug therapies for MMM are largely palliative and to date have not shown to significantly improve survival (3,6). Drug therapies may alleviate the associated anaemia, for which androgens, corticosteroids, and erythropoietin are the most effective first-line therapies (5). Such treatments bring a response in approx. 30% of patients with the response, often short-term, lasting approximately one year (5). Blood transfusion and regular folic acid therapies are also used in severely anaemic patients. Hydroxyurea remains the drug of choice for the control of leucocytosis, thrombocytosis (6), and in decreasing the rate of progression of splenomegaly (17), although busulfan (5), anagrelide (9), interferon alfa, cladribine (6), and low-dose melphalan have also proven to be effective in these areas (9). Experimental agents that inhibit collagen synthesis and fibroblast proliferation have so far failed to show significant clinical activity (17).

Thalidomide has recently been used with varying degrees of success in the treatment of MMM. Recent trials indicate that low-doses of the drug are effective and that the addition of prednisone improves drug tolerance and may enhance the erythropoietic activity of the drug (3,5). In addition, thalidomide appears promising for the alleviation of other disease-associated cytopenias (3). Thalidomide analogs with increased potency against TNF-$\alpha$ and decreased toxicity may allow prolonged exposure to the anti-angiogenic properties of the drug in the future (17). Other experimental trials involving drugs such as farnesyl transferase inhibitors and zarnestra, SU5416 (9), pirfenidone, imatinib and etanercept (5) , and pegylated IFN-$\alpha$ (21) are currently underway.

Splenectomy or splenic irradiation may be useful in selected patients (6,22). Splenectomy is considered in patients who are non-responsive to drug therapies, have splenic pain and/or discomfort, high RBC transfusion requirements, severe hypercatabolic symptoms, and symptomatic portal hypertension (6,8). Splenic irradiation may also be considered as an alternative treatment to splenectomy in those who are poor candidates for surgery. The benefits of splenic irradiation are transient with average response duration of six months (8).

Allogeneic stem cell transplantation (ASCT) provides the only possible curative treatment for patients (6,17,23). Until recently, the use of ASCT was discouraged because of the high mortality of 30% and the high risks of graft-versus-host-disease (5,6). In the past ASCT has often been restricted to cases with advanced refractory disease and considered only after failure of standard therapies (6). Today ASCT still presents high risks of morbidity and mortality, however, higher rates of post-transplant disease-free-survival are being reported especially in the younger age group. In these cases normalisation of the number of CD34+ cells in the circulation and a gradual normalisation of the BM fibrosis are reported findings (20). A combination of a younger age (<50), Hb > 100g/L, minimal symptoms of the disease, and <1% blasts in the peripheral blood are positive prognostic indicators for the success of ASCT (19,20).

Autologous stem cell transplantation is a palliative option for treatment, and a therapeutic possibility for refractory patients younger than 70 years of age who lack a suitable allogeneic marrow donor (23). The rationale behind the treatment is that bone marrow fibrosis and extramedullary haematopoiesis are processes related to tumour mass. Reduction in the tumour mass with the use of high-dose chemotherapy in combination with autologous marrow re-infusion may interrupt the disease process and allow time for healing by the harvested, mostly clonal, stem cells (6).

## Conclusions

MMM is a chronic progressive MPD disease with a poor prognosis, invariably resulting in the death of most patients following leukaemic transformation, or other events associated with reduced numbers of formed elements of the blood. For the majority of cases palliative care is the only option, with ASCT currently providing the most likely option for a cure of the disease. The success of ASCT as a treatment is limited to patients with good prognostic indicators and there remains a significant treatment related mortality associated with these processes. Most recent developments in research into the disease have been in to the molecular mechanisms of the disease. From this a greater understanding of the role of growth factors is beginning to allow a better understanding of the aetiology of the disease. At the genetic level progress has been slower and to date there remains little understanding of the gene defects that cause the disease. Studies into cytokines implicated in this disorder has lead to the development of several potential treatments for the disease. Trials of drugs targeting angiogenesis, tumour related cytokines, receptor tyrosine kinases, and intracellular transduction pathways are progressing and may lead to the release of new therapeutic agents for the treatment of MMM in the future.

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New Zealand Journal of
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# 60 Years of Continuous Publication Journal Prize

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

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

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

# A guide to the diagnosis of porphyria: suggested methods and case examples

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## Abstract

The objective of this review is to highlight the importance of the laboratory in the diagnosis of porphyria by providing the correct screening/diagnostic tests and offering interpretation of results.

Porphyria (por-fi're-ah) is due to a disturbance of porphyrin metabolism, characterised by marked increases in the formation and excretion of porphyrins or their precursors. Two main clinical manifestations may occur together or separately, potentially life-threatening attacks beginning with abdominal pain, and/or photosensitization of the skin. As the porphyrias may present in ways that mimic other disorders, and because different types share identical clinical features, accurate diagnosis requires the correct selection, performance and interpretation of laboratory tests.

An overview of porphyria is given, and several screening tests are discussed. Case examples are used to highlight the diversity and complexity of this group of diseases.

**Key words:** Acute intermittent porphyria (AIP), variegate porphyria (VP), hereditary coproporphyria (HCP), erythropoietic protoporphyria (EPP), porphyria cutanea tarda (PCT), congenital erythropoietic porphyria (CEP), porphobilinogen (PBG), Aminolevulinic acid (ALA).

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## Introduction

The porphyrias are uncommon disorders of haem biosynthesis and their effective management requires prompt and accurate diagnosis. Porphyria may present:

1. As an acute attack with abdominal pain and/or neurological symptoms.
2. With photosensitive skin lesions, skin fragility, erythema or bullae, leading to scarring and pigmentation.
3. As a combination of 1 and 2.
4. In the latent phase, e.g. as a relative of a known patient, or a patient presenting with a history of porphyria-like symptoms.

This article describes methods for the determination of urinary porphobilinogen, urine and faecal total porphyrins, and porphyrins in erythrocytes, as suitable tests for the use in non-specialist laboratories. It also highlights the need for accurate screening tests that are followed up with definitive diagnostic tests. A similar article written by the same authors has recently been published in the *New Zealand Medical Journal* (1), and focuses on the clinical indicators for the investigation of porphyria rather than the laboratory methodology of this article.

### Why is diagnosis the laboratory's responsibility?

The porphyrias form a heterogeneous group of inherited or acquired disorders of haem biosynthesis, and they are often missed or wrongly diagnosed, either as a result of being overlooked by the medical clinician or by incorrect or inappropriate laboratory testing. A partial deficiency of one of the seven enzymes in the haem biosynthetic pathway causes characteristic clinical and biochemical features. These disorders are due to specific alteration in the pattern of accumulation of porphyrin and porphyrin precursors (Figure 1). Each type of porphyria is defined by a unique pattern of accumulation and excretion of these haem precursors, as well as a reduction in the relevant enzyme activity and an associated genetic mutation (in all types except acquired PCT). Correct interpretation of the appropriate biochemical investigation is essential for accurately diagnosing and managing the porphyrias, as clinical features alone are not sufficiently specific either to confirm a diagnosis or to distinguish between the various forms.

Analogies can be drawn between this group of diseases and that of the current meningitis epidemic sweeping the country. Meningitis with its flu like symptoms can only be accurately diagnosis by the use of biochemical and microbiological investigation of cerebral spinal fluid. Clinically its early symptoms are misleading and often assumed to be flu. Similarly the type of porphyria can only be defined by detailed biochemical investigations.

Raised total porphyrins in the presence of skin lesions don't necessarily indicate a diagnosis of PCT, and it is the laboratory's responsibility to make this known to the referring clinician.

The following case demonstrates how misleading porphyria can be:

A 25-year-old lady of Pacific Island descent was initially seen by her general practitioner and found to have blistering and fragile skin on sun exposed skin. She was referred to a dermatologist for further investigation. She had no other significant medical history, but gave a family history of PCT. A presumptive clinical diagnosis of PCT was made. Fortunately, a full biochemical investigation was then undertaken. Raised urinary PBG, high performance liquid chromatography (HPLC) analysis of urine and faeces and the presence of a specific plasma fluorescence peak indicated acute VP. Genetic analysis identified a previously unreported mutation. Mutation and biochemical analysis was offered to the extended family; some of who had been assigned a diagnosis of PCT (probably erroneously without biochemical porphyrin studies), though to date this offer has not been accepted. If a full biochemical investigation had not been undertaken this lady would have been labelled along with the rest of her family as PCT, a much different disorder, where the patient does not suffer from potentially life-threatening acute attacks.

### What to test for:

Various testing scenarios have been published (2), but these usually rely on the examining clinician having a good knowledge of porphyria and how it may present. This unfortunately is not usually the case, as not only are the presenting conditions extremely variable, and most clinicians would be lucky to see one presenting case in their professional lives, due to their relative rarity. PCT the most common form of porphyria has an estimated incidence of 1 per 25,000 people; AIP, 1-2 per 100,000; HCP, EPP&VP, 1 per 250,000 (3). The authors therefore suggest the full profile of samples:

- ***Urine:*** Fresh random urine is preferred to 24-hour collections;

this is ideally collected during an acute episode if acute porphyria is suspected.

- ***Faeces:*** A random 10 g sample of faeces is required.
- ***Blood:*** Whole blood (EDTA or heparinised).

Specimens should be protected from light and received by the laboratory within 24 hours. The collection and processing of all three sample types eliminates the need for repeat visits to GPs and allows for a quicker concise diagnosis. If an acute porphyria is suspected, but a patient does not have current symptoms, sample request forms should be sent home with the patient, to be acted on when symptoms return. During latent porphyria some or all screening tests may be normal.

The following case demonstrates how easily porphyria may be overlooked:

A 52-year-old lady of South African descent was taken by her family to an accident and emergency department with severe and unexplained migraines. She had no history of migraine and was extremely distressed. After a full examination, no obvious cause could be found, though the severe pain abated after analgesia. She was admitted for observation overnight and discharged into the care of her general practitioner the next day, no diagnosis was made. Her GP, also South African, thought of VP as a possible cause. A full laboratory investigation was undertaken; this found raised levels of ALA, PBG, total urine and faecal porphyrins, and a diagnostic plasma fluorescence peak. Confirmatory mutation analysis detected the p. R59W mutation. Follow-up found that the patient's mother and two sisters had some years earlier been diagnosed in South Africa with VP, and that her acute migraine episode occurred several days post dental surgery anaesthetic.

VP is relatively common in the South African white population, estimated at 3 per 1000. New Zealand has over the last years had a large influx of immigrants from South Africa: 14,727 new immigrants between 1996 and 2001 (Census 2001 statistics), a 130 % increase. Therefore, laboratories around the country should be seeing the associated diagnosis of as many as 40 new VP cases.

**Peripheral lab tests:**

***Urine Porphobilinogen (PBG) screen.***

PBG is raised in patients during or in the days following an attack of acute hepatic porphyria, such as AIP, VP or HCP. PBG levels may return to normal between attacks (latent phase) or remain slightly raised. A normal level of PBG in urine collected during an acute attack of abdominal pain (the most common clinical indicator of an acute porphyria) excludes porphyria as a cause of the abdominal pain. PBG is never raised in PCT or EPP.

*Resin method:* pH adjusted urine is introduced into a syringe containing an anion exchange resin (AG1-X2 200-400 mesh, Bio-Rad). PBG in the urine binds to the resin on mixing, interfering coloured compounds are ejected from the syringe through a 5 μ filter. The resin is washed with water, then the PBG is eluted with acetic acid into a tube containing modified Ehrlich's reagent (0.7 g paradimethylaminob enzaldehyde dissolved in 150 mL conc. HCL). A pink colour is produced if PBG is present in abnormal levels (4).

This method overcomes the major problems of the Watson-Schwartz method, (5) namely, interference in the colour reaction by coloured compounds in the urine. This method is sensitive to about 20 μmol/L of PBG, independent of the colour of the urine.

***Urine total porphyrins.***

Porphyrins have characteristic electronic absorption spectra with an intense maximum around 400 nm that is known as the Soret peak. Very fresh urine specimens will contain up to 50% porphyinogens, which do not absorb at 400 nm. Conversion from the porphyrinogens to the porphyrins can take about 4 hours or more in urine but the rate is increased in acid. Therefore, for fresh samples it is best to dilute the urine in acid and leave it in the dark for an hour before scanning.

*Spectrophotometric method:* 1 mL of urine is diluted with 4 mL of 2.8 mol/L hydrochloric acid; this is then scanned between 385nm and 425nm. The height of the Soret peak at about 400nm reflects the total porphyrins present in the urine. Their concentration can be estimated using a combined molar absorptivity of coproporphyrin and uroporphyrin (6).

Urine can be a complex mixture of porphyrins and therefore an accurate measure of the total porphyrin is not possible without knowing the relative amounts of the various porphyrins. However, these problems do not affect the clinical usefulness of this assay, any abnormal results should be followed up with an investigation by HPLC.

*Reference interval:*

Urine total porphyrins: < 300 nmol/L

Urine porphyrin/creatinine: < 35 nmol/mmol creatinine (4).

Concentrations will be increased in patients with current symptoms of PCT, VP, HCP, AIP and CEP.

***Faecal total porphyrins.***

Faecal porphyrin concentrations are increased in hepatic porphyrias: PCT, HC, VP, EPP, gastrointestinal bleeding and very high meat diets, but not in AIP.

*Spectrophotometric method* (6): A small sample of faeces is homogenised in concentrated hydrochloric acid and then extracted with ether to remove interfering coloured compounds. On addition of water, coloured compounds, such as carotenoid and chlorophyll derivatives, remain in the ether phase whereas porphyrins partition into the acid aqueous phase. The aqueous phase is then scanned on the spectrophotometer in the same manner as that used for the urine samples. There is a direct correlation between the Soret peak height and the concentration of coproporphyrins and protoporphyrins. These can be expressed as concentration per dry weight of faecal material.

*Reference interval:*

Faecal total porphyrin: < 200 μmol/kg dry weight of faeces (4).

Biochemical laboratories around the country are rapidly becoming fully automated, as a result of which most laboratories would no longer be performing manual tests that would require spectrophotometry. The measurement of urine and faecal porphyrins should be used to justify the maintenance or replacement of a spectrophotometer along with other urgent tests, such as: CSF xanthochromia and urinary VMA.

***Whole blood.***

Red blood cell porphyrin levels may be raised in a number of types of porphyria (EPP, CEP, HEP and VP), but they may also be raised in a number of non-porphyria related conditions. In lead poisoning, ferrochelatase activity is inhibited and results in the formation of zinc protoporphyrin; in anaemia, resulting from iron deficiency, there is insufficient iron to create haem, thus zinc protoporphyrin is once again formed.

*Front surface illumination method* (Buchler Hematofluorometer): Light from a tungsten halogen lamp is directed through a 424 nm interference filter onto the bottom surface of a slide carrier, the slide carrier holds three slides, two of which are fixed within the instrument. These are the background check slide and an internal reference; the third slide holds oxygenated blood. As the operator moves the slide carrier through the instrument, each slide is exposed to the excitation light for 2 seconds. Re-emitted light from each of the slides is focused through another filter onto a photomultiplier tube. The photomultiplier measures the intensity of the background, reference and sample fluorescence, and from this the concentration of

**Figure 1.** The haem biosynthetic pathway shown in bold, the type of porphyria (or disease) shown in italics, the enzymes that catalyse the pathway shown on the right.

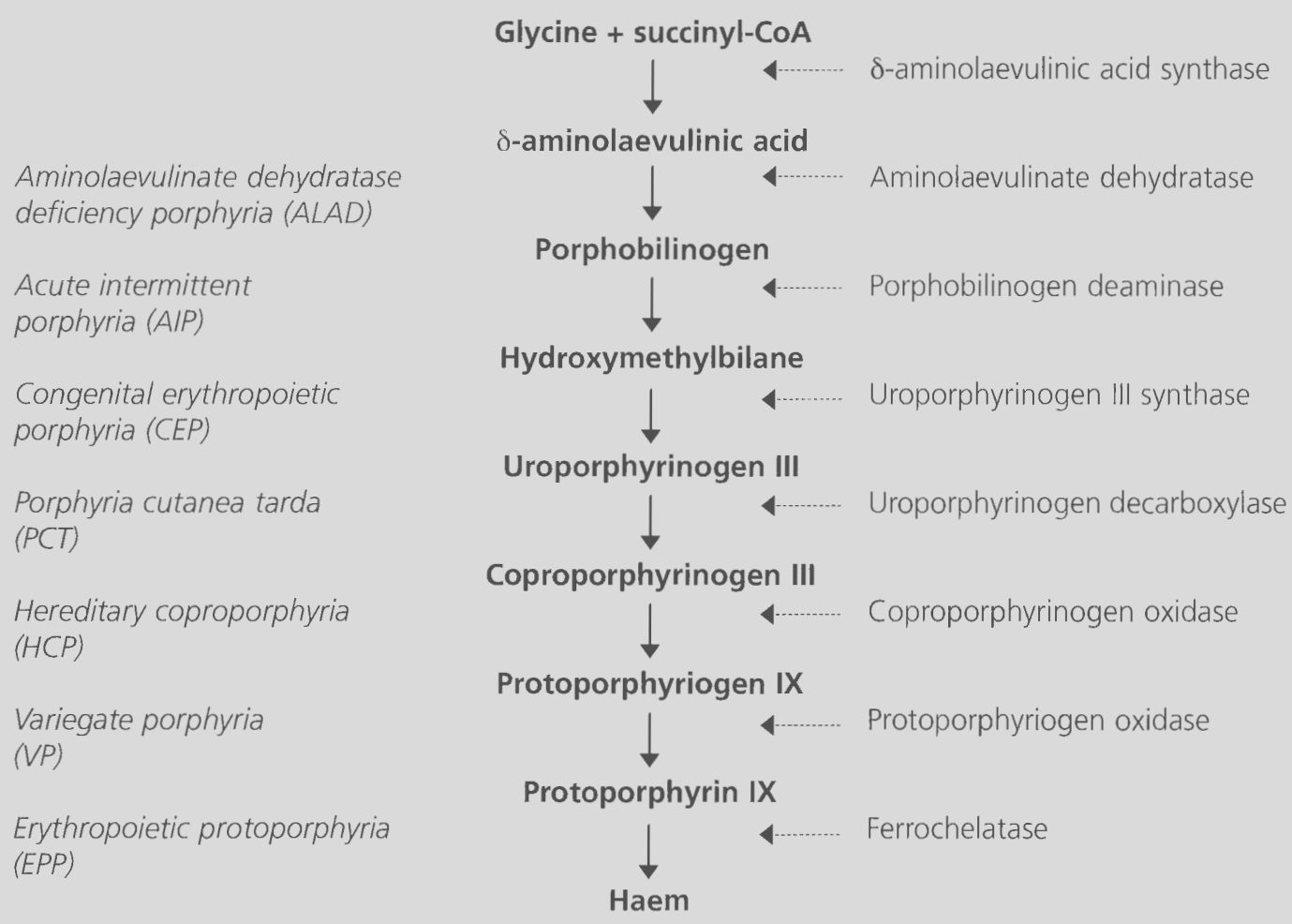
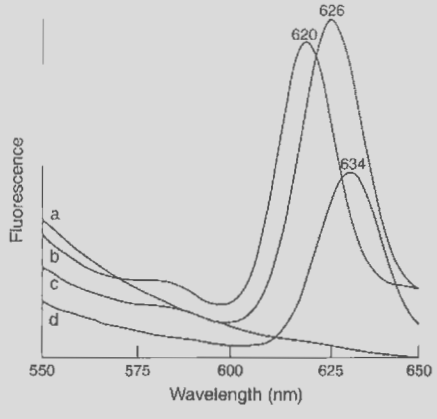



**Figure 2.** Fluorescence emission scans (excitation at 405 nm) of diluted plasma from (a) a normal subject and patients with (b) porphyria cutanea tarda, (c) variegate porphyria, or (d) erythropoietic protoporphyria.



erythrocyte protoporphyrin or zinc protoporphyrin can be calculated.

*Reference interval:*

Zinc protoporphyrin: 6-40 μg/100 mL.

Erythrocyte protoporphyrin: 6-36 μg/100 mL.

Alternatively, there are plasma and whole blood total porphyrin methods available using fluorometric methods, and spectrophotometric methods for total porphyrins on whole blood (4).

In the event that any of the above screening tests are raised or positive, all three samples should be referred to a reference laboratory that can perform quantitative analysis of urinary ALA and PBG, high performance liquid chromatography (HPLC), urine and faecal samples, plasma fluorescence scanning (see Figure 2), enzyme and or mutational analysis. Raised screening test results must not be considered diagnostic of porphyria or used to classify a certain type of porphyria.

The following case emphasizes the need for all three sample types:

The index case: a 6-year-old male was referred to a paediatrician after what the GP described as *"remarkable photosensitivity on minimal light exposure"*. By the time the child was referred and seen by the paediatrician, urine and faecal screening tests were normal, but erythrocyte protoporphyrins were raised at 40 (g/100 ml (6-36). Follow-up investigation showed slightly abnormal HPLC patterns on urine and faeces, and a specific plasma fluorescence peak consistent with EPP.

This presentation of photosensitive burning pain on sun exposed hands and feet are typical of EPP, as are the normal urine and faecal screening tests. Often, raised blood erythrocyte protoporphyrins are the only marker of this disease.

**Quality assurance.**

It is good laboratory practise to participate in external quality assurance schemes. This is not only an ISO requirement, but also possibly the only time when a laboratory will have the opportunity to become familiar with positive specimens. RCPA-AACB provides a good scheme for total urine and faecal porphyrins and a semi quantitative analysis of PBG. Internal quality assurance material is only routinely available for urinary porphyrins from organisations such as Bio-Rad or Chromsystems.

**The acute porphyrias.**

The three most common acute porphyrias, AIP, VP and HCP, are inherited as autosomal dominant disorders with clinical presentation after puberty. Many patients who inherit the enzyme abnormality remain asymptomatic during their lifetime. Abdominal pain is the commonest symptom of an acute attack. Nausea, vomiting, constipation, neuropathies and psychiatric symptoms may also accompany this. Hormonal changes (including menstrual cycle), drugs (lists of safe and unsafe drugs are available) and nutritional factors may aggravate the disorder (7). The skin symptoms of VP and HCP are: bullae, hyperpigmentation, and increased skin fragility. These abnormalities are due to accumulated free porphyrins in the skin, which absorb light and photodynamically damage cells.

**The rare acute porphyrias.**

The three rare porphyrias are congenital erythropoietic porphyria (CEP), hepato- erythropoietic porphyria (HEP) (the homozygous dominant form of type II PCT), and aminolevulinic acid dehydratase (ALAD) deficiency. All may present in childhood, and for CEP and ALAD are autosomal recessive.

Approximately 1 % of acute attacks of porphyria may be fatal (5). Most patients experiencing an acute attack will require admission to hospital, where only drugs known to be safe in porphyria should be prescribed. Oral or intravenous glucose and haem arginate is the mainstay of treatment. They inhibit synthesis of ALA, resulting in clinical and biochemical remission. Long-term, patients should be educated in the precipitating factors and should wear a Medic Alert bracelet. They should also be given an information booklet and/or encouraged to consult a support group web site (9,10).

**The non-acute porphyrias or cutaneous porphyrias.**

The two non-acute porphyrias seen in New Zealand are PCT, which probably accounts for 80% of all cases of porphyria, and EPP. PCT may be familial (autosomal dominant) or acquired. The disease may be precipitated by a number of factors including: excessive alcohol consumption, oral contraceptives, Hepatitis C, and haemochromatosis. The skin symptoms seen in PCT are indistinguishable from those of VP and HCP. Clinical symptoms of EPP however, include a sense of burning, oedema and itching, and are comparable to a severe case of sunburn. EPP is also autosomal dominant and usually presents in early childhood.

The cutaneous porphyrias are treated by the avoidance of sunlight and barrier protection. Additional options are venesection to deplete excess iron stores and oral chloroquine to increase urinary porphyrin excretion. For PCT, the avoidance of alcohol and oestrogens is also suggested. For EPP, red cell porphyrins and liver function tests should be checked every 6 months, as about 10 % of patients may develop liver disease and about 4 % die of rapid and fatal liver failure (11).

**Family studies.**

The importance of investigating relatives of patients with AIP, VP and HCP can hardly be over-emphasised. Every effort should be made to identify gene carriers before they develop symptoms, so that they can be warned to avoid known precipitants of acute porphyria. The first priority is the accurate diagnosis of the index case. This may involve enzyme tests and or genetic mutation analysis in addition to the full profile of tests discussed earlier. With this knowledge, the rest of the family can be investigated, beginning with the siblings and parents. The family is then followed from the oldest generation down. If a person is shown not to have inherited the enzyme defect, then their descendants need not be tested. These family members can be confident that they do not have porphyria. For young children (pre-puberty), gene analysis is the only reliable method of confirming or excluding porphyria, as even the enzyme assays are unreliable for this age group. Furthermore, the other porphyria screening tests are unreliable in this age group and should not be used. If porphyria is suspected, a full family study should be undertaken. Testing the urine of a 2-month old baby because great grandmother "had porphyria" can neither support nor disprove a diagnosis of porphyria.

The following case highlights a scenario where family studies should be undertaken.

A 39-year-old female was biochemically diagnosed as having AIP. On investigation her mother had previously (1970's) been diagnosed as VP, presumably incorrectly. However, she has managed to avoid any acute episodes and is still in good health at age 60. The index case's maternal grandfather and great aunt both died relatively young at ages 42 and 36 respectively, quite possibly from the complications of porphyria. There are 8 other family members who would benefit from a full family study, including the index case's young child.

## Conclusions

A clear concise diagnosis of an index case presenting with porphyria is essential. This then allows for follow-up family studies that can identify at risk family members. Those that are excluded of carrier status avoid the need for screening of future generations of that particular family branch. The input of a reference laboratory with experience of all the detailed procedures is invaluable.

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# LABORATORY AIDS IN THE DIAGNOSIS AND PROGRESS OF TUBERCULOSIS

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This paper is based on the experience gained in laboratory work done for the Pukeroa Sanatorium during the past 21/2 years. This Institution is functioning as a Sanatorium, and not as a chest hospital. Cases are admitted from the whole of the North Island area, and consist of people who have been recommended as suitable for sanatorium treatment. Most of them have passed through a general hospital prior to admission.

This paper sets out the routine laboratory procedure adopted in the primary investigation and follow-up of these cases.

## Sputum:

1. The *Direct Examination*: On admission, each patient has a direct examination of the sputum on six successive days. The report consists of:

1. The microscopic appearance of the sputum.

2. A report on the number of pus cells present as determined by microscopic examination of the stained slide.

3. The presence or absence of tubercle bacilli with a rough classification of the numbers present. Slides are reported as Positive or Negative, and the number of bacilli are classified as:

   Rare: 1 or less per 10 H.P. fields.

   Few: 1 - 10 per H.P. field.

   Numerous: 10 or more per H.P field

The microscopic appearance and report on the number of pus cells is of great value to the clinician. Specimens are classified as:

1. Purulent.

2. Muco-purulent.

3. Mucus.

4. Saliva.

A series of Negative reports on specimens consisting solely of mucus or saliva is obviously not as valuable as negatives obtained on specimens of purulent or muco-purulent nature. Of all direct examination positives, about three-quarters were obtained in purulent or muco-purulent material.

Cases producing purulent or muco-purulent sputum, and Z.N. Negative by direct and more sensitive methods, are worthy of further investigation to determine the presence of secondary infection, especially if the case is clinically and radiologically not typical of tuberculosis.

The Z.N. stained film is prepared in the usual way, great importance being attached to the selection of a satisfactory portion of the sputum. Variations of the original Z.N. method have been tried, such as counterstaining with picric acid, but our experience has been that there is no great advantage in adopting another method. This is probably a matter of personal preference, as we found that a picric acid counterstain was more trying on the eyes, when long periods have to be spent at the microscope.

Direct films are searched for a minimum of five minutes. During this time 500 microscopic fields can be thoroughly searched, and it is emphasised that the Direct Examination is purely a "screening out" test for fairly obvious Positives, the main purpose being to classify the cases into Direct Examination "Positives" and "Negatives". It is then necessary to further investigate the Negatives, and unnecessary concentration work is avoided on the Direct Examination Positives.

In addition to the six direct examinations on admission every patient has one monthly direct examination of the sputum for T.B. This is of value for two reasons:

First, the cases which have been consistently positive, if responding to treatment, will eventually become negative on the monthly direct examination. This is the first laboratory indication of progress, and these patients then have six direct examinations, on successive days. If these all prove negative then the investigation proceeds to more sensitive methods for the detection of the tubercle bacilli. Second, it is of value in cases previously classified as Negative by direct examination, to determine if they remain negative by this method. It can happen that a case which has been negative may regress and produce a positive sputum, and this can sometimes be picked up in the monthly examinations.

At the discretion of the Medical Officer, any case may be investigated by means of six direct examinations on successive days, at a time when the doctor notices that the patient is producing an increasing amount of sputum. This increase may be due to any one of a number of reasons, e.g., the common cold if prevalent throughout the Institution will often result in a marked increase in the number of cases found positive by direct examination.

*Concentration and Culture Methods:* (three-day collection of Sputum). Cases which have been Negative by direct examination on admission, or have become negative after being positive, are investigated by means of Concentration and Culture Methods. It is not proposed to enter into details of the technique of such methods, beyond stating that we have used the Petroff and Hank's Alum precipitation methods for concentration, with satisfactory results.

Culture Media used and found satisfactory are Lowenstein, Yolk enriched (Edson), modified yolk enriched, and Petragnani. For simplicity combined with satisfactory growth, modified yolk enriched is an excellent medium. From the description of the direct examination procedure it will be seen that cases proceeding to concentration methods are those producing either very few tubercle bacilli or else none at all. Therefore it is obvious that the concentration picture, as we find it, will not be so spectacular as that shown in a laboratory where all sputa are submitted to concentration without prior "screening out" of obvious positives. The point of importance here is that concentration films must be searched very thoroughly, and our procedure is to search films for 15 minutes before reporting as "Negative." Films are frequently found "POSITIVE" after 10 minutes or so of concentrated searching, and after the long time involved in the concentrating and preparation of the film it is obviously important to give really adequate time to the microscopic examination. Quite apart from the importance of the result to the patient and clinician, a hurried "Negative" report will be followed by further concentration involving a considerable amount of work, which possibly could have been avoided by adequate searching of the original film.

A case giving a positive concentration result will not have further concentrations for some little while - it will depend on his clinical and radiological progress. Those cases giving negative concentration results will be followed up with further concentrations at the discretion of the Medical Officer concerned. The concentration method is of definite value in two ways:

1. It does determine a considerable number of positives which, although suspected on other grounds, would be bacteriologicallly negative.

2. Repeated Negative concentrations are much more reliable results than those obtained by direct examination only.

*CULTURAL METHODS*: All concentration deposits are cultured. Culturing is a more sensitive method again than examination of concentration films. We culture all positive concentration deposits, for the purpose of typing the tubercle bacilli. The negative deposits are cultured to attempt to obtain positive results, and those that are positive are typed.

Cultures have yielded positive results in numerous cases when concentration films have been negative, and we have not yet failed to grow tubercle from a deposit which has been positive in the concentration film. Practically all the tubercle cultivated has been of "human" type. This can be explained by pointing out that most of the cultural work is done on cases of pulmonary tuberculosis. Where there are lesions other than in the lungs, they are usually secondary to a lung condition.

The only "bovine" types encountered have been in hospital cases and not from the Sanatorium.

*GASTRIC CONTENTS:* The examination of the Gastric contents is an important point in the detection of the tubercle bacilli. This method is employed in cases which are producing minimal amounts of sputum, or none at all. When very small amounts of sputum are produced there is a tendency for this sputum to be swallowed instead of expectorated. Children are particularly likely to do this, and some adults will also swallow considerable amounts of sputum. The resting stomach contents are collected by means of a stomach tube on two mornings. The resulting fluid is concentrated and cultured in a similar manner to that employed for three-day sputa.

This is a particular useful line of investigation for children and non-co-operative patients. We have found it an advantage to prolong the period of digestion with NaOH and use exclusively a medium containing malachite green for culturing gastric contents. This seems to obviate difficulties encountered due to contamination of gastric contents cultures with organisms resistant to the NaOH treatment.

## Animal Inoculation:

This examination is run parallel with cultures, but cannot be done routinely on all concentrations, as this would involve an extremely large amount of work and a very large supply of animals. Inoculations are, therefore, restricted to selected cases: first, cases that have some clinical evidence of T.B. and have not yielded a positive by other methods,: and, secondly, in cases that are nearing the point of discharge from the Sanatorium. As a point of interest, and bearing in mind that the animal inoculations only follow after repeated attempts at obtaining positives by other means, we find that animal and culture results are running fairly even.

## Faeces:

The examination of faeces for tubercle is undertaken:

a) As an alternative to examination of the gastric contents.

b) To aid in the diagnosis of tuberculosis of the bowel.

It is a more tedious procedure than examination of the gastric contents, and our experience has been that it yields no more information than gastric concentration.

With regard to the diagnosis of T.B. of the bowel, it is difficult to come to a conclusion as to the significance of T.B. in the faeces. In open cases of pulmonary T.B., tubercle bacilli can be demonstrated in the faeces without there being any clinical evidence of bowel involvement, and have presumably been from swallowed sputum. In a case where Gastric contents are repeatedly negative, and the faeces positive, then possibly some deductions can be made. Material obtained directly from a lesion in the bowel will give a more reliable result than examination of the faeces. However, in young children, where there is difficulty in obtaining sputum or gastric contents, the examination of the faeces is of value.

## Pleural Effusions:

You are all quite familiar with the usual methods of examining pleural fluid for T.B. The only point to be made here is the extremely satisfactory results obtained from the culture of pleural fluids, but it is necessary to examine large amounts of the fluid, up to 100 ml. being necessary for satisfactory examination. The few c.c. of fluid so often submitted for examination is very often inadequate, and over the last 11/2 years, since we have introduced the method of repeatedly examining large quantities of fluid, we have not failed to establish the bacteriological aetiology in any Sanatorium case with pleural effusion.

A point of interest is that in the "Lancet," February 9, 1946, an article points out that it is necessary to aspirate large amounts of fluid to obtain good results, and this article also emphasises the superiority of cultural methods over animal inoculation. The cytology of pleural fluids is to large a question to be dealt with here, and possibly will be the subject of a later article, but the cytology of the pleural fluid is by no means predominantly lymphocytic at every stage, as is often stated. There seems to be a definite connection between the polymorph lymphocyte ratio and the later demonstration of tubercle bacilli, bur much more work remains to be done on this subject.

An easy and fairly accurate estimation of the increasing or decreasing cellularity of a pleural fluid can be obtained by centrifuging a specimen in a graduated centrifuge tube for a fixed time at a fixed speed. The deposit is reported in terms of "percentage sediment." It gives more definite information when combined with the differential count of the cells than does the differential count alone, and the Sanatorium medical officers have found this simple test to be of some value.

## The Mantoux Test:

Again, this test is quite familiar to you all. The only point of interest is that it is sometimes overlooked in the investigation of possible cases of T.B. A negative result is of definite value. In Sanatorium work old tuberculin is preferred to P.P.D. as the great difference in 1st and 2nd strength P.P.D. (2nd strength 250 times stronger) can result in too severe reactions in dealing with Sanatorium cases. Old tuberculin in dilutions 1 in 100,000, 1 in 10,000, 1 in 1,000 and sometimes 1 in 100 is used.

## The Blood:

*The Sedimentation Rate* (S.R.): The most widely used test is the S.R. We use the Wintrobe method. Each patient has a monthly S.R. and it is used chiefly as an indication of progress rather than as a diagnostic test. Cases similar clinically will often yield very varying S.R's, and most importance is attached to comparative studies of the S.R. Correction for anaemia is done on cases showing a S.R of over 20 mm. in 1 hour. Both observed and corrected rates are reported and the clinician makes his own interpretation. It is still regarded as a useful laboratory procedure.

## The Examination of the Peripheral Blood:

This is done only in selected cases. During 1944 all Sanatorium cases had the blood examined as follows:

a) Differential white count.

b) Arneth count.

c) von Bonsdorff count.

d) Houghton's index.

As far as we know this was the first extensive work done in N.Z. on the blood changes in active or recently active tuberculosis. The work was done on the lines set out by Houghton in his original article in "Tubercle," November, 1935, and we refer you to this article for full details.

The results of a year's work, entailing many thousands of blood examinations, was that the blood picture is of definite value in prognosis.

*Conclusions:* Progress can be accurately assessed and clinical breakdown anticipated by serial blood examinations. The blood picture is also of assistance in selecting cases for special treatment.

## The Urine:

All patients have a routine laboratory examination of the urine on admission. Early cases of renal tuberculosis have been picked up by this means. Any pathological feature reported in the original examination ensures that a more extensive investigation will follow.

The 24-hour specimen is not recommended as a means of demonstrating tubercle. We prefer to examine the last specimen at night and the first morning specimen. This is more satisfactory for cultural work, and from the point of convenience. Usual concentration methods are adopted. T.B. in the urine is now regarded as always being pathological, even in the absence of clinical features. The theory of "excretory bacilluria" is no longer tenable, and serial sections have demonstrated kidney lesions in cases of "symptomless tuberculous bacilluria."

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of the

NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS

Volume 1. April. 1946 No.1

# EDITORIAL

The commencement of a Journal is never a step to be undertaken lightly, especially when subjects of a scientific nature are to be dealt with. However, it was the unanimous opinion of those present at the first Annual General Meeting of the Association, held in Wellington, that a Journal was a necessity as a means of keeping all the members of the Association acquainted with the progress of their fellow members and the dissemination of all knowledge thought to be of interest and use.

The progress of the Journal and its value will, however, depend on the active support of all members, senior and junior, for material to publish, for constructive criticisms and suggestions, and in the initial stages for a generous allowance for difficulties in publication.

At the present it is intended to make this a quarterly journal and to print it in Auckland with the assistance of members of that Laboratory. Should this venture prove a success, the problem of providing a suitable press will have to be faced and a discussion on the Journal and its future should be a subject for consideration at the next Annual General Meeting. In the meantime the Editor would be grateful for suggestions, notes, articles and references for the next issue, these to be in hand by June 1st for the July issue.

# TO THE EDITOR

Dear Sir,-

On behalf of the Council and Members of the Association I extend congratulations on your good effort in the production of the first issue of the *Journal*. I wish to endorse the remarks contained in your first editorial, and would urge all members, senior and junior, to give their active support to the Journal.

Our second Conference will be held in the near future and we look for the keenness and co-operation that characterised the first Conference. We are now an established body with a membership of 75. We are also an Incorporated Society, but I feel that we must once again discuss the question of registration in the light of knowledge and experience gained since the deputation approached the Director-General of Health. Following representations from our Association, the Director-General of Health has advised us that "the person qualified and holding the Department's Certificate was to be known as a 'Hospital Bacteriologist'," but I notice that in the Social Security (Laboratory Diagnostic Services) Regulations 1946, para. 5 (b) such persons are still referred to as "bacteriological assistants."

I still seek support to the idea of a preliminary examination to provide for recognition of experience gained in laboratories at present not under a Pathologist.

I strongly urge that CONFERENCE 1946 will have strong representation from all laboratories.

Yours sincerely,

E.L.F. BUXTON, President

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# Book Reviews

| | |
|---|---|
| **Title:** | Modern Blood Banking and Transfusion Practices |
| **Author:** | Denise M Harmening |
| **Edition:** | 5th |
| **Publishers:** | F A Davis, Philadelphia |
| **Date of Publication:** | 2005 |
| **ISBN:** | 0 8036 1248 6 |
| **Price:** | NZ$ 181.15 |
| **Available from:** | Elsevier Australia. www.elsevier.com.au |


Modern Blood Banking and Transfusion Practice is the 5th edition in this series by Denise Harmening. The author is from the School of Medicine, Department of Medical and Research Technology, University of Maryland and is a former medical laboratory scientist, now academic. Professor Harmening has built a strong reputation in the USA as an educator, researcher and administrator in medical science. Dr. Harmening is the author of three major textbooks: *Clinical Hematology and Fundamentals of Hemostasis, Modern Blood Banking and Transfusion Practices*, and *Laboratory Management*. In the preface the author states the book is a culmination of the input of over forty contributors that make up the 27 chapters of the latest edition. Three new chapters covering: *Concepts in Molecular Biology, Information Systems in the Blood Bank, Medicolegal and Ethical Aspects of Providing Blood Collection*, and *Transfusion Services* have been included in this edition. The book has been written primarily for medical laboratory technicians and scientists, blood bank specialists and residents, and provides a concise and thorough guide to transfusion practice and the science of immunohaematology.

As mentioned, contributions to this new edition have been gleaned from those working in transfusion medicine in the USA. The book is therefore biased toward technical practises in the USA which are not dissimilar to those used in NZ. This edition presents the basics of transfusion medicine with coverage of immunology, genetics and molecular biology in addition to the usual blood groups, and infectious diseases spread by blood transfusion. The book is structured so that the basic principles of transfusion medicine are presented early thus allowing the reader a greater understanding of the chapters presented later in the book.

The book commences with a chapter on the historical aspects of blood transfusion, blood preservation, the metabolism of red cells and platelets and a brief review of progress with alternatives to red cell and platelet transfusions. The principles of genetic inheritance, cellular and molecular genetics and DNA typing techniques are covered along with the fundamentals of immunology to include immune suppression and responsiveness, and the diversity of the immune response in humans. This edition has a new chapter on molecular biology which sets the text apart from many others with a similar readership. In this chapter DNA function, repair, cloning, recombinant DNA, restriction enzymes, gel electrophoresis, DNA fingerprinting and PCR, help to provide blood bankers with a base of knowledge in the subject. The chapter on the antiglobulin test compares DAT's using both gel and tube methods and is followed by a chapter on the ABO blood group system which provides adequate coverage the now well-established biochemical polymorphisms that lead to the ABO groups. The Rh blood group system is presented in a similar fashion so as to provide a basic level of understanding of the systems antigens and antibodies. The other blood group systems and the many antigens and antibodies of these systems are presented in a manner that does not swamp the reader with the mountain of information that is available on the blood groups.

The book takes the reader through the processes of donor selection and deferral in the USA, accreditation testing, product manufacture and usage and apheresis. It also covers alloantibody testing, gel techniques, titrations, compatibility testing including the computer crossmatch and an authors view into the future of pretransfusion testing. Other chapters of the book cover the adverse effects of blood transfusion and includes the laboratory investigation of HTR and DHTR. A chapter on transfusion transmitted diseases is presented covering the established hepatitis viruses, HIV, HTLV-I/II as well as current eg. WNV and other agents that in the future may become of greater concern in blood transfusion. This section also covers the emerging technologies of viral inactivation, quarantine, recipient tracing and prion diseases.

As with any good transfusion medicine text this book would not be complete without coverage of haemolytic disease of the newborn and foetus, autoimmune haemolytic anaemias (includes a useful section on absorption and elution techniques), and the HLA system. Blood groupings, HLA antigen typing and RFLP analysis of DNA, for parentage testing is covered in a separate chapter and the section on quality management in the blood bank presents material that will be familiar enough to most blood bankers in NZ. Between references to the FDA and other US accreditation authorities, NZ readers should be able to pick up the overall message of the importance of a quality systems approach, one that applies equally in the NZ setting.

The section on transfusion safety and federal regulatory requirements and the final chapter on medicolegal and ethical aspects of providing blood collection and transfusion services are perhaps more for the administrators among the profession. While each make an interesting read, both have little relevance in NZ. The second to last chapter covers information systems in the blood bank and is sufficiently generic to provide some background for NZ Progesa users.

The things I liked most about the book were the layout of the chapters which allowed for a logical progression through the subject. I also liked the structure of the chapters in which learning objectives were set and at the end a summary of important points and a range of short answer questions to test your level of understanding were provided. I also liked the inclusion of a chapter on molecular biology, an area too often omitted from similar texts. My only criticisms of an otherwise excellent textbook are that I felt the coverage of the biochemistry and cellular function of the blood groups lacked a certain depth, particularly for the ABO and Rh systems. In some sections, particularly with the blood groups, there was a breakdown of the material presented into basic and advanced or specialist knowledge something I felt of questionable benefit.

The book commences with a foreword by Dr. George Garratty in which he states that this latest edition by Harmening contains in a single volume all the information necessary for the training of medical laboratory scientists in the medical specialty of transfusion medicine. This is strong support from Dr. Garratty who authors his own texts on the subject and who has for many years also taught medical scientists.

My overall impression is that this is a book written by blood banker professionals for blood bankers. As such it has real relevance to the transfusion lab and transfusion medicine in general. As the author states, the book has been produced as a training textbook primarily for scientists and technicians and is not intended to be a complete reference textbook. This edition will therefore have wide appeal to those training of scientists and technicians in NZ and as such would make a useful addition to the bookshelves of blood banks throughout NZ.

***Chris Kendrick MZNIMLS, Grad Dip Sci, MSc (Dis), Senior Lecturer in Haematology and Transfusion Science.***
***IVABS, Massey University, Palmerston North***

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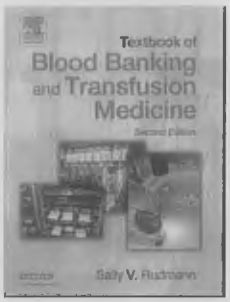
**Title:** Textbook of Blood Banking and Transfusion Medicine
**Author:** Sally V. Rudman PhD, MT(ASCP) SBB,CLS
**Edition:** 2nd
**Publishers:** Elsevier Saunders, Philadelphia, USA
**ISBN:** 0 7216 0384 X
**Price:** NZ$ 113.65
**Available from:** Elsevier Australia. www.elsevier.com.au




This book has been written primarily for students of medical laboratory science as a text book rather than a scientific or technical reference. It contains both basic and more advanced concepts which make it suitable also as a text for those undertaking technician training. The book was written as the author states, to *"sequence material in such a way to organise learning and provide a logical approach to the content"*.

**General overview of the book**

It is well set out making it user-friendly for the reader. Some of the features of the chapters are:

- Important terms in the text appear in the margins with a quick reference which can be useful as a guide to study.
- Messages found throughout the chapters as margin notes, help to reinforce important concepts or to act as reminders and cross references to other related information.
- "Expert opinions" are scattered throughout the book providing relevant and interesting advanced information.
- Each chapter commences with an outline and a summary of important points which are a good guide to the content of the chapter.
- Objectives and expected learning outcomes for the student are provided with each chapter.
- Multi-choice questions related to the learning objectives of each chapter are provided at the end of the book and are useful self assessment exercises.
- References and further readings are found at the end of each chapter.

**Chapters content**

Comprehensive coverage of fundamental genetics and basic immunology pertaining to blood banking are provided in the first two chapters. The ABO and Rh Blood groups are well covered with the biochemistry of the secretor status for both the ABO and Lewis systems presented in a manner that can be easily understood. Other blood groups appear together in a single chapter and are covered appropriately in less detail as compared to the ABO and Rh systems. Chapter six also provides an interesting section on veterinary transfusion medicine.

The chapter on the human leucocyte antigens (HLA) seems comprehensive and covers the basics well. It also has sections on transfusion related acute lung injury, solid organ and bone marrow transplantation, paternity testing, and disease associations.

In the chapters covering donation, preparation and storage, much valuable information is presented about donor screening. This was, however, very specific to practice in the United States of America and I felt placed too strong an emphasis on the donation phase of blood collection. The text does refer to the American Association of Blood Banks (AABB) standards, which are also used as a guide to practice in NZ. There is a good section on autologous, directed and apheresis donations.

The section on blood components is well covered and discusses the merits of whole blood and fresh blood transfusions. The book recommends differing temperatures and storage times for thawed fresh frozen plasma, and other frozen components from those used in NZ. There is a good section on the preservation and storage of blood components and an interesting expert opinion on the public's response to blood donation in the aftermath of the twin tower attack in New York.

The section on compatibility testing presented new advances in the use of barcode patient identification systems. Samples receipt acceptance and storage were also covered. This section of the text included a large section on the administration of transfusions by non-laboratory staff which I felt was not required. The section also provided in-depth information on blood filters, intravenous infusion devices and rates, pressure devices, patient monitoring, etc.

The chapter dealing with blood group antibody identification was one of the best. This contained many examples of alloantibody identification panels that blood bankers will appreciate. It outlined a useful approach to exclude antibodies in the process of assigning alloantibody specificity and also covered haemolytic transfusion reactions and auto-immune haemolytic anaemias. A discussion about whether we should worry about not detecting antibodies to low incidence antigens concluded the chapter.

Technical problems and anomalies that arise with blood grouping were covered in the next section. This too was excellent, with examples and resolutions for many problems encountered in the lab. Each was presented in a case study format.

A component therapy section provided details on the selection of blood components and blood product usage. In this section indications for the use of each were provided, as well as the use of autologous donation and intraoperative salvage blood. Also in this section, is a discussion on the use of erythropoietin in patients electing autologous transfusion for surgery.

The chapter on adverse effects is a very comprehensive with a good section on the pathophysiology of haemolysis. Disease transmission is also well covered, as is the investigation of suspected transfusion reactions.

Haemolytic Disease of the Newborn provides a number of detailed illustrations, however, I felt the section on amniocentesis was too detailed, being better suited to medical specialists rather than blood bankers.

Massive transfusion, the treatment of coagulopathies with blood components and the outcomes and adverse effects of transfusion are covered in a single chapter. The options for the use of plasma exchange for different disorders are presented, as are the use of transfusion in burns patients, liver and renal patients. The section on newborn transfusion was perhaps presented in too great a detail, and was again better suited for other medical specialists.

The last few chapters contained good coverage of the autoimmune and drug-induced haemolytic anaemias, and comprehensive coverage of haemopoietic stem cells and cellular therapies. There was also a very good expert opinion provided on cord cell banking.

Finally I found that the safety, quality and record keeping chapters of the book were too detailed in areas, particularly the quality chapter which should have been referenced to a quality manual. The section on record keeping was too specific to the American blood bank to be of particular use to us in NZ.

Overall, I feel that this book would make a valuable reference text for the blood bank professional. It covers blood banking theory, technical practice, regulatory guide-lines, related transfusion medicine and at times provides advanced concepts for the specialist. The book was extremely interesting to read and the layout excellent for study purposes. A feature I particularly liked was the use of highlighted text throughout the book. These were printed in red directing the reader to margin notes that explained key points in the text. Illustrated tables and charts are used to good effect throughout the book.

In comparison to the AABB technical manual, which is often used as a training and reference text in NZ, I felt that this book was better set out and more user-friendly. Although written for blood bankers in the USA, I think that students and existing blood bankers will find this a useful addition to the collection of texts in NZ blood banks.

***Bronwyn Kendrick, MNZIMLS, Medical Laboratory Scientist.***
***NZBS - Manawatu, Palmerston North.***

# New products and services

## Hettich centrifuges spin in to Airpro

One of the most important pieces of laboratory equipment in use today is the humble centrifuge. Whether it is used for scientific, medical or industrial purposes, its ability to operate reliably is crucial. From separating blood cells to discovering new drugs and other materials, the centrifuge is an important piece of every scientist's equipment.

But it is not just a case of filling the vials, letting the machine spin up and seeing what happens next. Good centrifuges have to spin at a reliably known rate, for a known time and often at a known temperature; if these things don't happen, then not only can an important experiment or other process be ruined, but also laboratory time and expensive materials can be wasted as well.

Airpro Scientific Ltd. was pleased to be able to add Hettich centrifuges to complement the company's focused suite of high quality world recognised brands. Although Airpro approached Hettich initially (because they have been manufacturing quality centrifuges for 100 years), Hettich recognised the growth potential for their products in the New Zealand market fitted Airpro's growth strategy.

This is an important development for Airpro, as despite Hettich centrifuges until recently having had multiple distributorships in this country, sales have not been great. This has probably been, as Airpro's Business Development Manager Trevor Clark believes, the reason that no one has really exploited the product's potential.

"Hettich centrifuges are extremely well-known and very highly regarded in laboratories around the world", Trevor recounted recently. "Indeed many scientists who come from overseas to work in this country are surprised they are not more readily available".

"We have been appointed by Hettich because they know Airpro Scientific has a culture of excellence and also that we have the resources to make the most of this opportunity", Trevor continued. "We are going to ensure that every scientific, medical and industrial laboratory in New Zealand hears about Hettich centrifuges and that they have the opportunity to understand the benefits they deliver".

Trevor concluded by saying that Airpro Scientific was delighted by this development as it also enabled the company to products that were truly amongst the best in the world.

"With Hettich centrifuges alongside the other excellent products we offer, I think we can confidently look forward to significant growth in the near future and to achieving our goal of being the leaders in our field".

# Med-Bio Journal Award

med·bio

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

# Journal-based questionnaire

Below are 10 questions based on articles in this issue of the Journal. The articles can be original, editorials, letters or review articles, so read the entire issue of the Journal. The questions are in the format of true/false.

Mail or fax your answers, to be received no later than 30 April, to the NZIMLS Executive Office, PO Box 555, Rangiora; fax: (03) 313 2098. Please include your email address.

You must get at least 7 questions right to earn 5 CPD points. You will be notified once the Editor and Deputy-Editor have checked your answers.

***Circle your answer or delete accordingly.***

Among the chronic myeloproliferative diseases, MMM is the most prevalent.

**True** **False**

In the final phase of myelofibrosis, a progressive decline of the WBC can be seen.

**True** **False**

Clonal cytogenetic abnormalities occur in approximately 50% of patients with MMM.

**True** **False**

Conventional drug therapies for MMM are largely palliative.

**True** **False**

In 1973 colour advertisements were published in the Journal for the first time.

**True** **False**

To celebrate 60 years of the Journal, a special prize of $500 is offered for the best case study published during 2006.

**True** **False**

A normal level of PBG in urine collected during an acute attack of abdominal pain excludes porphyria as a cause of the abdominal pain.

**True** **False**

Porphyrins have characteristic electronic absorption spectra with an intense maximum around 500 nm that is known as the Soret peak.

**True** **False**

Raised screening test results are considered diagnostic of porphyria.

**True** **False**

The three most common acute porphyrias, AIP, VP and HCP, are inherited as autosomal dominant disorders.

**True** **False**

Full name:.....

Laboratory/organisation: .....

City/town:.....

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# MICROBIOLOGY SPECIAL INTEREST GROUP

## DUNEDIN
## FRIDAY 28TH AND SATURDAY 29TH APRIL 2006

**FRIDAY AFTERNOON (2 - 5PM)**

- What you don't know about antibiotics
- Choosing and using antibiotics - a pharmacy perspective
- *Clostridium difficile* - new developments
- Applications, Implications and use of oral Streptococcus as probiotics

**FRIDAY EVENING (7 - 9PM)**

- Unusual infections in renal patients
- Endotoxin testing in Haemodialysis patients

**SATURDAY (9 - 5PM)**

Proffered papers

**Registration information and further details to follow soon at www.nzimls.org.nz**

# New Zealand Institute of Medical Laboratory Science

# INAUGURAL NORTH ISLAND SEMINAR

## HAMILTON
## KINGSGATE HOTEL
## SATURDAY 13th MAY 2006

**In conjunction with**
**THE NZ FLOW CYTOMETRY GROUP MEETING**
**(Friday 12th and Saturday 13th May)**

The inaugural North Island Seminar will be held at the Kingsgate Hotel in Hamilton.

All presenters (from all disciplines) are most welcome to submit. Presentations will be allocated a 15 minutes maximum timeslot. Prizes for best overall paper and best first time presenter.

Post seminar buffet dinner and fabulous entertainment from Austin Powers (International Man of Mystery) and the Legends Cabaret.

Accommodation has been reserved at the Kingsgate for Saturday 13th May. Some also available for the night of Friday 12th.

Requests for proffered papers and registration information attached and available online at the NZIMLS website (www.nzimls.org.nz)

For additional information please contact either:-Robin Allen allenr@waikatodhb.govt.nz (07) 839 8636 or 021 244 4837 Tony Mace Tonym@pathlab.co.nz (07) 858 0799

For details of the Flow Cytometry Group Meeting :– Barbara Harrison harrisb@waikatodhb.govt.nz.

# Histology Special Interest Group Meeting

**Venue :** Wairakei Resort, Taupo

**Date:** 4th November

**Convenor:** Dr Joe McDermott - LabPlus, Auckland Hospital

The Histology SIG is scheduled for the 4th of November at the Wairakei Resort in Taupo. This is the first call for papers and posters - if you wish to participate in the academic programme please send an abstract to Frances Murray at FrancesM@adhb.govt.nz. A prize for the best presentation and poster is up for grabs!

Further updates and a provisional outline of the day will be sent out to the Histology mailing list - if you wish to join the mailing list please send an email to Steve Cooke at scooke@adhb.govt.nz.

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# HSIG questionnaire

**Haematology**
Special Interest Group

Journal article questionnaire for the Haematology Special Interest Group.

"Why should women have lower reference limits for haemoglobin and ferritin concentrations than men?" British Medical Journal Vol. 322, 2 June 2001, p.1355-7.

Questions:

1. The role of iron in humans is central to what process?
2. When does the haemoglobin concentration of women revert to that of age matched men?
3. What percentage of UK females of childbearing age does not achieve the recommended daily dietary iron intake?
4. The recommended daily dietary iron intake is.
5. Where does haem synthesis occur?
6. What products in the typical UK diet can limit iron absorption?
7. An increase in what is related to irritability in iron deficient children?
8. Besides reduced red cell parameters, what are some other consequences of iron deficiency for the body? Name 5
9. At what age is the full adult iron complement of the brain achieved?
10. What proposal is made in this paper regarding the "normal" values for red cell count, haemoglobin and ferritin levels?
11. What suggestion is made to rectify assessment of iron status in women?

Prepared by Jacquie Case, Section Head, Special Haematology, Middlemore Hospital. For a copy of the journal article, Ph 09 276 0044, ext 8515. E-mail: jcase@middlemore.co.nz

Answers on page 44

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# The Pacific Way

On the 26 October 2005 the Pacific Paramedical Training Centre celebrated its 25 year Jubilee at a reception held in the Wellington School of Medicine. Attending the function were old and new friends of the PPTC including many Medical Laboratory Scientists, some who travelled from out of Wellington to attend.

Mr John Elliot Director of the PPTC welcomed the guests and invited Mr Peter Adams, Executive Officer NZAID, Dr Ashley Bloomfield from the NZ Ministry of Health and Dr Ron Mackenzie, Chairman of the PPTC to give short addresses.

John Elliot also read a letter from Dr Ken Chen, WHO Representative to the South Pacific. His letter is printed below:

**Remarks for the 25th Anniversary of the PPTC, 26th Oct 2005, Wellington, New Zealand.**
**Dr Chen Ken. WHO Representative to South Pacific**

"Our Hosts - John Elliot, Director of the PPTC and all PPTC staff members, New Zealand Government Representatives, Friends, Distinguished guests, ladies and gentlemen.

Thank you very much for the invitation to the World Health Organization to be present at today's celebration. It gives me great pleasure to be able to address you all at this auspicious occasion and to congratulate the PPTC on its round 25th anniversary. I am really sorry that I am not able to join all of you physically today due to several urgent issues which I need to be involved including working with Pacific island countries in preparing influenza pandemic preparedness plan.

The PPTC is our valuable, staunch and long-term partner in the Pacific region. Our collaboration over the years has been extensive, fruitful and, we firmly believe, beneficial to the health systems of the Pacific Island nations. The fact is best signified by the PPTC's status of WHO Collaborating Centre and its recognition by many other partners. But first and foremost, it is the appreciation by the Pacific island countries Governments, and by the multitude of laboratory workers in the Pacific Islands Health Laboratories. I think there is no laboratory worker in the Pacific, who wouldn't have received training in some form, who wouldn't know PPTC trainers from their numerous country visits, and who wouldn't have worked hard on the PPTC Regional External Quality Assessment samples, trying to get the correct results.

But it is not only the official activities by the PPTC, which makes it a truly regional institution. It is also the personal touch by the PPTC staff members, your friendliness, open-mindedness, cross-cultural approach and ability to built harmonious personal relations with all the partners in health in the Region.

By using this opportunity, I would also like to express our sincerer thanks to New Zealand government, New Zealand Ministry of Health and NZAID for your support to WHO's efforts in Pacific such as prevention of non-communicable diseases, tobacco control, promotion of physical exercise and healthy diet, mental health and water safety. PPTC has embraced the idea of distance learning and continuous professional education. Several courses for the WHO Pacific Open Learning Health Net have been developed by the PPTC. Several more laboratory POLHN courses are under development or planned to be developed in collaboration with the PPTC. One of the major needs is to ensure accreditation of the PPTC and POLHN courses.

We face some big challenges in the province of laboratory services in the Pacific, for instance:

- Continuous quality improvement in all areas of laboratory work.
- Preparedness for new emerging diseases.
- Support for the prevention, control, treatment and monitoring for the diseases gaining prominence in the countries' disease profiles, such as NCD, HIV infection/AIDS.
- Implementation of new technologies.
- Strengthening of the laboratory public health functions - e.g. food water, environmental samples analysis.
- And many others.

We have to be ready to face and tackle those challenges. With this in mind I wish the PPTC happy 25th birthday and another at least 25 years of productive and beneficial work in the Pacific. WHO looks forward to continue its collaboration with the PPTC."

Dr Ron Mackenzie and Dr Sandy Ford, who were the founders of the PPTC and who developed the concept of short term training courses for Pacific Island Laboratory Technicians, cut a celebration cake.

Past and present staff, tutors and committee members of the PPTC who attended the 25 year Jubilee celebration.

**Distance learning courses**

In December 2005 the PPTC signed an agreement with the WHO POLHN (Pacific Open Learning Health Net) to develop and teach a basic course in Medical Laboratory Technology. This new course will include models in Microbiology, Biochemistry, Haematology, Blood Bank Technology and Immunology and will lead to a Certificate in Medical Laboratory Technology granted by the PPTC. The course will take a full academic year to complete and is aimed at giving the theoretical background to supplement the practical training which technicians receive in their laboratories. Each module will be of 5-6 weeks duration and will consist of weekly PowerPoint lectures, a practical laboratory log book plus means of assessing each student's progress. The first modules, Biochemistry and Haematology will be available in the second half of this year and further information will be circulated to all laboratories in the Pacific shortly.

# Index to Volume 59, 2005

# Massey University - NZIMLS prize for 2005

The winner of the award in 2005 was Nicole Keegan from Massey University. Nicole received the sum of $2000.00 from the Institute to assist her with the completion of her BMLSc at Taranaki Medlab this year. Nicole was selected for the award as the top academic student in the 300 level year of the Massey BMLSc programme.

Born in New Plymouth Nicole is the youngest daughter of Gerard and Gayle Keegan, and grew up with brother Jarrod and sisters Rochelle and Tania. Nicole has lived in Taranaki most of her life, attending school at Saint John Bosco Primary, Highlands Intermediate and Sacred Heart Girls' College. In 2002 Nicole was granted a Taranaki Medlab Scholarship, and began the BMLSc degree at Massey University in Palmerston North in 2003.

Throughout childhood and into adult life Nicole has been a keen sportsperson, with particular interest and enthusiasm for golf. She has enjoyed many successes including being the Senior Sportsperson of the year at Sacred Heart College in 2002 and was a member of the Manawatu/Wanganui Senior Women's' Representative Golf team from 2003 to 2005. Most recently Nicole captured the Manawatu/Wanganui Champion of Champions women's golf title. Nicole's future interests and goals include completing her degree, travelling and perhaps furthering her education at some point in time.

The NZIMLS congratulates Nicole on her award and wishes her all the best for the year ahead and her future career.

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New Zealand Journal of
Medical
Laboratory
Science

# NZIMLS Fellowship

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

Rule 3.17 of the Fellowship Regulations states:

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

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

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

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

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

***Robin Allen, Rob Siebers, Ann Thornton. Fellowship Committee.***

# Abstracts of presentations at the NZIMLS ASM, Christchurch,

**Inflammatory bowel disease: how bed to beside research has translated to improvements in patient assessment and drug treatment**

***R Gearry. Christchurch Hospital and School of Medicine and Health Sciences, Christchurch***

Inflammatory bowel disease (IBD) comprises Crohn's disease and ulcerative colitis. Research shows that IBD incidence has increased exponentially in recent years. Symptoms include abdominal pain, diarrheoa, rectal bleeding and weight loss. The diseases usually have their onset in young people but have no cure, leading to a relapsing-remitting pattern of symptoms. Calprotectin, a protein found in the cytoplasm of neutrophils, is found in all body fluids in proportion to the degree of inflammation. Measurement of faecal calprotectin (FC) provides clinicians with a non-invasive but sensitive means of assessing intestinal inflammation. Clinical applications include differentiating organic and functional gastrointestinal disease, and predicting whether or not IBD patients will relapse in upcoming months. Faecal calprotectin provides clinicians with a non-invasive window to the gastrointestinal tract. The thiopurine drugs azathioprine and 6-mercaptopurine (6-MP) are the most effective drugs available for maintaining remission in IBD patients. They also play a central role as immunosuppressants and steroid-sparing agents in autoimmune diseases such as rheumatoid arthritis, autoimmune hepatitis and in the setting of solid organ transplantation. However, these drugs have a complex metabolism with marked inter-patient variability. Recent developments have allowed clinicians to be guided by phenotype and genotype assays of the enzyme thiopurine methyl transferase (TPMT). Additionally, measurement of 6-thioguanine nucleotides (6-TGNs) has allowed clinicians to ensure patients are prescribed the correct thiopurine dose. Research is being translated from bench to bedside, improving care for patients with inflammatory bowel disease.

**Clinical chemistry and renal disease**

***C. Florkowski. Canterbury Health Laboratories, Christchurch***

Estimated GFR - Glomerular Filtration Rate (GFR) can be estimated from plasma creatinine using the Cockroft and Gault formula (*Nephron* 1976; 16 :31-41), although requires height and/or weight to be supplied. An alternative equation is the Modification of Diet in Renal Diseases (MDRD) equation (*Ann Intern Med* 1999; 130: 461-70), which does not require height or weight. GFR (mL/min/1.73m2) = 186 X [creat (mmol/l) / 0.0884]-1.154 X [age]-0.203 X 0.742 (if female) The MDRD equation was validated in a group of predominantly Caucasian males with chronic kidney disease. It has not been validated in children (aged <18 years), pregnant women, the elderly (aged >70 years) and ethnic groups other than Caucasians or African Americans. Although both prediction equations perform acceptably well when compared with gold-standard radio-nuclide GFR, on average the MDRD equation is more accurate and precise (www.kidney.org/professionals/doqi). Reference range: 80 -120 mL/min/1.73m2, though GFR declines by 1mL/min/year over age of 40 years.

Cystatin-C is a 13.4 kDa protein. Given that it has constant production by all nucleated cells, is freely filtered at the glomerulus and catabolised by proximal renal tubular cells, it is an ideal marker of GFR. Immunoassays are available for Cystatin-C with constant ranges that are independent of height, age, gender and body composition (*Clin Biochem* 2005; 38: 1-8). Serum Cystatin-C starts to increase as GFR falls below 80 ml/min compared with 40 ml/min for creatinine. Despite this, there is not a strong evidence base for improved clinical outcomes, although it may assist in the earlier diagnosis of renal transplant rejection (*Clin Chem* 1999; 45: 2243-9).

Proteinuria. The trend is away from timed urine collections with guidelines favouring 'spot' urine samples for the detection of microalbuminuria (albumin : creatinine ratio >3.5 g/mol), macro-proteinuria (>22.7 g/mol: *Ann Intern Med* 2003; 139: 137-47) and for the prediction of albuminuria >300 mg/day in pre-eclampsia (>30 g/mol: *Br J O&G* 1997; 104: 1159-64). Recent studies have also demonstrated the presence of a modified form of albumin not detected by conventional immunoassays (*Kidney Int Suppl*; 2004: (92): S65-6). This 'ghost' albumin is an earlier marker of microalbuminuria in diabetic patients and has implications for assay design and the interpretation of key studies undertaken to date.

**Coeliac disease**

***M Spellerberg. Canterbury Health Laboratories, Christchurch***

Diagnostic testing for Coeliac disease is widely available in New Zealand laboratories and employs techniques as varied as Indirect immunofluorescence and Luminex technology. Although the sensitivity and specificity of these assays is high, there are patients who give equivocal results. Currently they will be referred for endoscopy to confirm characteristic changes to bowel morphology. If DNA typing for the HLA 'risk' alleles in coeliac disease is performed, patients who do not carry these alleles could avoid endoscopy.

**Coeliac disease and diabetes**

***J Willis. Christchurch Hospital, Christchurch***

The prevalence of coeliac disease is higher in individuals with type 1 diabetes, than in the general population. Coeliac disease and type 1 diabetes share an autoimmune aetiology and are associated with the same susceptibility alleles of the HLA locus. Further, diabetes-related autoantibodies may be present in 25% of coeliac patients, and may predict future development of type 1 diabetes. Individuals with type 1 diabetes often have silent or atypical coeliac disease, which is manifest only after screening for coeliac-related antibodies. Coeliac disease contributes to growth impairment, osteoporosis, reproductive problems, and malignancy. In addition, diabetic individuals are at increased risk of hypoglycaemia due to malabsorption. Screening individuals with type 1 diabetes, and first degree relatives, for evidence of coeliac disease is indicated.

**Breast cancer genetics**

***C M Morris[1], L C Walker[2], G C McKenzie[2], B A Robinson[1]. Christchurch School of Medicine & Health Sciences[1] and Christchurch Hospital[2], Christchurch***

Breast cancer, like other cancers, is a genetic condition that is

caused by changes or "mutations" in DNA, the molecule that encodes the building blocks of all of the cells that make up our body. Some women inherit and are predisposed to develop the disease because of genetic changes that are passed on from parent to daughter. However, most breast cancers (> 90%) are caused by genetic changes that are acquired during our lifetime as an individual, occur specifically in breast tissue, and are found only in the cancer cells. Internationally, research is showing that analysis of the genetic profile of tumour cells taken at the time of biopsy can tell us a lot about the disease, how it will grow and the likelihood of future spread. The ultimate hope is that some of these genetic changes will be highly predictive of prognosis, and that they will also determine the types of treatment to which the tumour will best respond. However, much more research is needed to confirm and extend these early findings, and to develop simpler and less costly methods that will allow detection of the most important genetic changes in a routine diagnostic laboratory setting. Towards this end, we are applying DNA copy-number profiling in combination with high throughput microarray analysis in a prospective program of research designed to better understand the biological basis that determines observed differences in histopathological characteristics of breast cancer and which may have prognostic relevance. To date, for example, our comparative genomic hybridisation (CGH) analysis of 41 core needle biopsy samples has identified a region of copy number variation on chromosome 8 that may associate with tumour grade.

**The molecular analysis of colorectal cancer**
***M Whitehead. Christchurch Hospital, Christchurch***

Hereditary Non-Polyposis Colorectal Carcinoma (HNPCC) is one of the familial bowel cancers that form a small but important subgroup of colorectal cancer. Unlike the polyposis syndromes HNPCC does not have macroscopic morphologic differences from sporadic colorectal cancer that can be used to identify the affected family members. The cancers usually occur at a relatively young age, are often multiple and affect multiple family members which can be devastating to a family if not screened. The molecular pathogenesis of these cancers is now reasonably well understood and with immunohistochemical markers we can identify a genetic defect to help identify these families further to allow screening and earlier detection of these tumours.

**Antivascular treatment of tumours**
***G U Dachs[1], M J Currie[1], G M Tozer[2], B A Robinson[1]. Christchurch School of Medicine & Health Sciences[1], Christchurch and University of Sheffield[2], Scheffield, UK***

A functional vascular network is essential for the survival, growth and spread of solid tumours, making blood vessels a key target for therapeutic strategies. Two new classes of anti-cancer agents specifically target the tumour blood vessels, namely anti-angiogenic agents, which interfere with the formation of new blood vessel, and anti-vascular agents, which target the existing tumour blood vessels. This presentation will give an overview of these classes, and then concentrate on three specific anti-cancer approaches, two of which are small molecule based anti-vascular drugs, and one is a novel gene therapy-based method. Combretastatin A-4 phosphate (CA-4-P), derived from the African bush willow, is a tubulin-depolymerising agent in Phase II clinical trials as a tumour vascular targeting agent. DMXAA (5,6-dimethylxanthone-4-acetic acid) was developed in New Zealand as a vascular disrupting agent and has recently entered Phase II clinical trials. Data on the molecular mechanism of action of these two compounds will be presented, as well as an overview of in vivo and clinical findings thus far. Gene therapy represents an alternative to current anti-angiogenic and anti-vascular approaches. It has the potential to provide a high level of specificity and hence minimises side effects.

**Platelet transfusions and the problem patient**
***K G Badami. New Zealand Blood Service, Christchurch***

Platelet transfusions are used to treat or prevent bleeding in thrombocytopenic patients and those with certain platelet function disorders. Prophylactic platelet transfusions are used to raise counts to 'safe' levels and thus prevent bleeding - mainly catastrophic, 'spontaneous' central nervous system or retinal bleeds. Patients unresponsive to such transfusions therefore constitute a serious clinical problem. However, it must be stressed that with platelet counts there isn't a clearly defined threshold of risk. Between 1960 and 1990, counts less than 20 x $10^9$ / L were the trigger for prophylactic platelet transfusions in stable patients. In those with sepsis or other complicating factors including those undergoing invasive procedures, higher triggers were, and continue to be, used. Since the 1990's, a lower trigger - 10 x$10^9$ / L or exceptionally, even 5 x $10^9$ / L has been used. As a matter of fact, there is some evidence that the platelet count on its own predicts future bleeds poorly and that bleeding during the previous three days may be a better indicator of further bleeds and the need for platelets. Platelet transfusions are used for supporting patients with both short episodes of thrombocytopenia (such as with DIC) but also, more importantly, those with thrombocytopenia of longer duration such as following cancer chemotherapy or stem-cell transplantation. The latter often require support for weeks or months and who are especially at risk of becoming refractory to random platelet transfusions with, potentially, great consequences to life and treatment costs. The clinical response to therapeutic platelets in bleeding patients and increments with prophylactic random platelets in non-bleeding patients are used to assess and establish refractoriness. Non-immune causes such as sepsis, splenomegaly and certain drugs should be considered and excluded. These account for the majority of cases of refractoriness (up to 80 % in some studies) and are amenable to relatively simple corrective measures. The remainder are presumably due to immune causes. Because platelets have ABO (but not Rh) antigens, major ABO-mismatched platelet transfusion (such as A group platelets to an O group patient) may be associated with poor increments - an immune cause that should be considered early in the work-up. Platelets also have HLA class I (but not class II) and 'platelet-specific', human platelet antigens (HPA). Alloimunization to foreign HLA and less commonly, HPA may cause immune refractoriness. However, primary alloimmunization requires that alloantigens are initially presented to CD4-positive T-cells by HLA class II-bearing antigen presenting cells (APC) such as dendritic cells. Leucodepletion of cellular components has reduced alloimmunization but not eliminated it - presumably because recipient APC are also involved in presenting alloantigens to recipient T-cells. Thus, the most important preventive measure against refractoriness is avoiding needless transfusions. From a practical viewpoint, often the initial step in dealing with the 'refractory' patient (i.e., one who has more than once failed to respond satisfactorily to an adequate dose of 'fresh', random, ABO-compatible platelets and in whom non-immune causes have been excluded) is to screen for HLA antibodies with the standard or anti-human globulin-enhanced lymphocytotoxicity (LCT) test. Broadly-reactive LCT positivity correlates well with platelet refractoriness. Such patients should receive HLA class I-matched platelets. HLA-matching includes identifying not only the best-matched donors but also those with nominal but cross-reactive and non-immunogenic mismatches. Refractory patients in whom HLA antibodies cannot be demonstrated with the LCT could either have a

trial of HLA-matched platelets (it is important that patients likely to be platelet transfusion-dependent for significant periods are prospectively HLA typed) or have other tests performed to detect anti-HPA antibodies. The solid phase red cell adherence assay (SPRCA) or the monoclonal antibody-specific immobilization of platelet antigens assay (MAIPA) can detect both anti-HPA and HLA antibodies and are therefore sometimes used as initial screening tests. Having defined the specificities to which the patient has antibodies, it may then be possible to choose platelets which are negative for the relevant antigens. Finally, platelet cross-matching using patient's serum with random donor platelets directly identifies suitable donors by testing for significant HLA, HPA and other differences. Thus, nominal but clinically insignificant mismatched donors referred to above are not excluded from the search. This is sometimes performed up-front and obviously, is useful for patients for whom suitable HLA-matched platelets cannot be found. Options for patients who fail to respond to all the above measures are currently uncertain and limited. A 'no-prophylaxis-treatment-only' platelet transfusion policy with perhaps, larger than normal doses when required, antifibrinolytic agents, immunosuppressive therapy, plasma exchange, 'HLA-stripped' platelets, cryo-preserved autologous platelets and platelet growth factors are among the many methods that have been used to deal with this difficult problem.

**Are our doctors 'trigger' happy?**
***C E Foley. Lakes DHB, Rotorua***

In order to determine whether Rotorua Hospital doctors are observing transfusion trigger guidelines (1), an audit of blood transfusion practices was carried out. Four months data were gathered from transfusion records held in the blood bank in conjuction with information obtained from the Clinical Information System (CIS). Data collected included pre-transfusion Hb, clinical diagnosis including relevant pre-existing conditions, and the service area involved. The data were sorted according to pre-transfusion Hb levels. Apparent non-compliant transfusions, according to NZBS guidelines, were identified. These were further investigated through the CIS and/or hard copy patient notes and some were subsequently reclassified. Preliminary results indicate that up to 9% of transfusions were not compliant with the guidelines.

In conclusion, up to 9% of transfusions at Rotorua Hospital were non-compliant with the transfusion trigger guidelines. 1. Is this an issue we need to address more regularly as we are unaware of these incidences on a day to day basis? 2. What level of non-compliance is considered acceptable? Communication and education of medical staff is an important function of our transfusion practice, particularly if we see compliance as a necessary part of everyday bloodbanking.

1. Transfusion Medicine Handbook 2003. New Zealand Blood Service, New Zealand.

**The neonate and their environment**
***M McIhone. Christchurch Womens Hospital, Christchurch***

In 2004 there were 755 admissions to the Christchurch Women's Hospital Neonatal Unit. Of this number 334 were less than 36 weeks gestation (76 of this number were less than 32 weeks gestation). The neonates entry and subsequent immediate experiences of extra-uterine life are fraught with difficulty and challenges, not withstanding the absence of his/hers mother's womb. The recent relocation of the Neonatal Unit to the new Christchurch Women's Hospital has allowed us to create an environment that addresses the preterms infant's developmental requirements that in the past were difficult to achieve because of the enforced physical surroundings that we had. During the immediate period after birth, no matter how much effort is directed at removing 'noxious stimuli' and replicating the intra-uterine environment, the preterm infant is always at risk from several potential life threatening conditions

1. Anaemia of prematurity
2. Hyperbilirubinaemia
3. Neonatal sepsis

Reference: Recommended standards for Newborn ICU Design, Consensus Conference on Newborn ICU Design, January 2002.

**Myeloperoxidase, oxidative stress markers and cardiovascular disease**
***C Winterbourn. Christchurch School of Medicine & Health Sciences, Christchurch***

There is accumulating evidence that oxidative stress is an important contributor to the pathology of cardiovascular disease. Oxidation of low density lipoprotein is one mechanism for lipid accumulation in atherosclerotic lesions. Oxidants can also cause endothelial dysfunction, plaque rupture and inactivation of proteins that are important for cardiac function. Diets high in antioxidants are associated with reduced cardiovascular risk. of the various of sources of reactive oxidants, NADPH oxidases, which are present on the endothelium, smooth muscle cells and cardiomyocytes, are particularly relevant to cardiovascular disease. These are activated by various stimuli, including angiotensin II, to generate superoxide radicals and hydrogen peroxide. Inflammation is also important, and recent evidence points to a role for myeloperoxidase (MPO), an enzyme present in neutrophils and monocytes. MPO's prime purpose is to convert hydrogen peroxide into stronger oxidants such as hypochlorous acid for killing pathogens, but these oxidants can also attack host tissue. MPO binds to endothelial cells and has been detected in atherosclerotic lesions. Associations between plasma MPO levels and both endothelial dysfunction and early risk of heart attack in patients reporting to a lipid clinic or with chest pain have been observed. We have studied heart failure patients for evidence of oxidative stress and its relationship to MPO. We found that plasma MPO levels were elevated but to a lesser extent than post myocardial infarction. Using protein carbonyls as an oxidative marker, we observed extremely high levels in plasma from these patients. While MPO appears to be useful for prognosis in some groups, it is not yet apparent whether oxidative markers such as protein carbonyls are useful predictors of outcome.

**Coenzyme Q10**
***S L Molyneux, C M Florkowski, M Lever, P M George. Canterbury Health Laboratories, Christchurch***

Coenzyme Q10 (CoQ10) is an essential cofactor in the mitochondrial respiratory chain responsible for oxidative phosphorylation. Furthermore, the reduced form has a primary function as an antioxidant. CoQ10 is lipophilic, and is therefore transported in blood lipoproteins. Some CoQ10 may be obtained from the diet, however the majority is endogenously synthesised via the mevalonate pathway. Cholesterol lowering statin drugs (HMG-CoA reductase inhibitors) lower endogenous synthesis of CoQ10, due to the common biosynthetic pathway of these two compounds. CoQ10 supplements are available, and widely sold, over the counter from health food shops and pharmacies in New Zealand. Supplement brands contain various formulations, including dry powder, oil suspensions, and oil plus surfactant emulsions. Supplemental CoQ10 has been reported

to improve health in many disease states, including lowering blood pressure, and improving quality of life in heart failure patients. We have set-up a method for measurement of plasma total CoQ10, utilising HPLC with electrochemical detection. We have estimated a reference interval for total CoQ10 in the New Zealand population, and calculated the biological variation of CoQ10 in healthy subjects. A comparison of the bioavailability of seven CoQ10 supplement brands available in New Zealand has shown that there is a significant difference not only in bioavailability of supplements, but also in absorption of CoQ10 by different volunteers. This indicates a need for monitoring of blood levels during supplementation, to confirm efficacy.

**UK national external quality assessment scheme for immunocytochemistry (UK NEQAS ICC)**
***Stephanie Neal. Canterbury Health Laboratories***

Immunocytochemistry is used extensively to assist in routine clinical diagnosis and is proving to be invaluable in the selection of patients for specific therapy. However, the quality of the immunohistochemical staining can vary greatly between different laboratories, which makes it necessary to combine both an internal and an external quality assessment programme. The UK NEQAS for immunocytochemistry began as a pilot scheme in the UK in 1984 and was recognised by the UK department of Health in 1988. Since then the scheme has grown tremendously and now has 583 participants, 250 of which are from the UK and 283 from 38 other countries including New Zealand, Australia and Hong Kong. The scheme is solely subscription financed and is a 'not for profit' organisation. The UK NEQAS ICC is divided into 7 immunocytochemistry modules: general pathology, breast pathology (hormonal receptors), breast pathology (HER-2), lymphoid pathology, neuropathology, cytopathology, and alimentary tract pathology. Very recently fluorescent in situ hybridisation module has been introduced for HER-2 (HER-2 FISH). Assessments take place approximately every 3 months and involve the combined efforts of 50 external assessors comprising of pathologists and biomedical scientists. Approximately 5000 slides are analysed within a 4-week period. Feedback is given to participants as individual assessment results along with the scheme journal (www.ukneqasicc.ac.uk), which incorporates the main technical parameters employed by all participants. A main aim of the scheme is to provide all participants, irrespective of geography, information on methods and reagents that allow for improved quality of immunocytochemistry with the intention of providing a basis for the standardisation of protocols. The process of assessment and changes being implemented will be further discussed using data taken directly from UK NEQAS ICC modules.

**Diagnosis of pneumococcal infection**
***Dr Murdoch. Canterbury Health Laboratories, Christchurch***

Streptococcus pneumoniae is a major cause of morbidity and mortality worldwide, is the most common cause of community-acquired pneumonia in both adults and children, and is also an important cause of meningitis, bacteraemia and otitis media. The definitive diagnosis of invasive S. pneumoniae infection is difficult to establish using conventional diagnostic tests, especially in children. The limitations of existing diagnostic tests have implications that extend further than for the routine diagnosis of S. pneumoniae infection in clinical laboratories. The assessment of region-specific disease burden and the effectiveness of public health interventions, such as pneumococcal vaccination, are also hindered by the difficulties diagnosing pneumococcal disease. Evaluations of pneumococcal PCR assays have produced variable results. Newer pneumococcal urinary antigen tests have shown considerable promise, especially for diagnosing pneumococcal pneumonia in adults. The clinical importance of the newly described species Streptococcus pseudopneumoniae remains unclear.

**Detection of enterobacteriaceae with decreased susceptibility to quinolones**
***M C Robinson, A J Jaksic. Medlab Bay of Plenty***

Salmonella serotypes with decreased susceptibility to fluoroquinolones are being isolated from humans and animals with increasing frequency and have been associated with treatment failures. Routine laboratory testing may not detect reduced fluoroquinolone susceptibility of these isolates, as minimum inhibitory concentrations of the fluoroquinolones are usually within the susceptible range of the interpretive criteria of the CLSI (NCCLS). A growing body of clinical and microbiological evidence indicates that resistance to nalidixic acid can be used as a surrogate marker of decreased susceptibility of Salmonella to fluoroquinolones and the CLSI (NCCLS) Performance Standard for Antimicrobial Susceptibility Testing of Enterobacteriaceae have included reference to the need for nalidixic acid testing when reporting fluoroquinolone susceptibility of Salmonella strains. We hypothesised that decreased susceptibility to fluoroquinolones could be also detected in other Enterobacteriaceae using the nalidixic acid susceptibility result. Nalidixic acid screening of Serratia marcescens isolates assisted in detecting reduced fluoroquinolone susceptibility. The same phenomenon has also been demonstrated in a *Citrobacter braakii* isolated from blood cultures. In vitro exposure of nalidixic acid-resistant (but ciprofloxacin-susceptible) isolates to ciprofloxacin resulted in the selection of ciprofloxacin-resistant subpopulations within 24 hours. Nalidixic acid screening is now performed on all isolates of Enterobacteriaceae in our laboratory.

**The therapeutic use of apheresis**
***P Flanagan. New Zealand Blood Service, Auckland***

NZBS is the primary provider of therapeutic apheresis services in New Zealand. This reflects the synergy with donor apheresis work. Overall demand for therapeutic apheresis services is static. Peripheral blood stem cell collection (PBSC) is increasingly preferred to bone marrow collection for use in stem cell transplants. PBSC is less traumatic for the donor or patient and provides a purer product with less red cell contamination. This reduces complications associated with ABO incompatibility in allogeneic settings. Autologous PBSC collection is increasingly undertaken. In the context of allogeneic transplantation stimulation of stem cell production requires the use of GSF or other growth factors. This can be used in related but not in unrelated donor settings. This issue is being progressed by the NZBMDR. Current challenges in this area relate to stem cell selection procedures, involving either negative selection (T-cell depletion) or positive selection (CD34 selection) and also the ability to safely collect stem cells from younger children. Plasma exchange continues to be used in a number of clinical disorders. The increasing use of intravenous immunoglobulin has reduced the need for plasma exchange in some diseases. Overall demand however remains stable. The availability of growth factors has led to increased interest in the area of granulocyte transfusion. Higher yields resulting from GSF stimulated donors improve the overall effectiveness of granulocyte transfusion. Overall demand remains low, this in part reflecting the success of modern supportive therapy with antibiotics and antifungal agents.

**Amphetamines, does your assay measure up?**
***S Patterson. Canterbury Health Laboratories, Christchurch***

Between 1998 and 2004 there has been a 200 fold increase in the number of 'P' labs cleaned up by ESR. 'Herbal highs', most notably benzylpiperazine, are freely available in shopping malls throughout suburbia. Cold and flu medication, containing pseudoephedrine, is a stock item in many home medicine cabinets. Which amphetamines do we need to be targeting and should we be tailoring our analyses to our clients needs? These are some of the questions that needed to be addressed in evaluating some of the current immunoassays available.

**Mysterious waxy deposit in urine**
***W Woltersdorf. Canterbury Health Laboratories, Christchurch***

A urine sample containing a waxy supernatant was sent for identification. It originated from a 63-year-old female patient, who had recently undergone gastro-intestinal surgery. Nursing staff denied use of suppositories or enemas. Initial investigations showed clear urine with a hard, paraffin-like substance attached to the plastic surface of the container. Sudan fat-stain was negative and microscopy was unremarkable. It did not dissolve in ethanol and X-ray diffraction did not detect any crystalline structures. The melting point of the material was 34°C, which was identical to the carrier used for paracetamol suppositories (Paracare™, PSM Healthcare). Thin layer chromatography also showed an identical migration pattern. Paracetamol recovery into acetaminophen-free human plasma was positive for the control suppository but negative for the index sample suggesting complete absorption in vivo. Subsequently, the patient confirmed the use of suppositories on direct questioning but this was not recorded in the case notes.

**Diagnosis of PNH by flow cytometry**
***L McArthur. Canterbury Health Laboratories, Christchurch***

Paroxysmal Nocturnal Haematuria (PNH) is a non-inherited stem cell disorder caused by mutation of the pig-a gene on chromosome X. Understanding of the molecular defect has led to a disease specific test for PNH, and flow cytometry is now considered to be the gold standard diagnostic technique for this disorder. We describe the mechanism of PNH as a clinical disease and explain how the diagnostic test works. Indications for requesting PNH testing by flow cytometry and sample requirements are also discussed. The patient in our case study had an unusual clinical presentation that occurs in less than 10% of PNH cases. The patient has a healthy syngeneic twin and will undergo bone marrow transplant as a potential curative therapy. Factors that provide PNH clones with a growth advantage remain poorly understood, and the opportunity to investigate PNH in twins may lead to better understanding of this rare condition.

**Survival markers in blood**
***B D Hock[1], J L McKenzie[1], W N Paton[1], M Albitar[2]. Haematology Research Group[1], Christchurch Hospital, Christchurch and University of Texas[2], MD Anderson Cancer Center, Houston, USA***

Newly diagnosed leukaemia patients vary considerably with respect to both the rate at which their malignancy progresses and their responses to therapy. There is therefore considerable interest in identifying the biological factors important in the development/progression of leukaemia as this information may (i) allow the development of new treatment options and (ii) provide new prognostic markers. The identification of improved prognostic markers allows more optimised treatment by identify both high risk patients and those that respond poorly to current therapies. The cell protein CD40 plays an important role in the development of anti leukaemia responses and reagents targeting CD40 are currently being investigated in clinical trials as leukaemia treatments. We determined in initial studies that a soluble form of this protein (sCD40) circulates at high levels in some leukaemia patients. We therefore analysed the clinical significance of sCD40 levels in leukaemia in order to determine whether they provide a useful prognostic marker and whether sCD40 may potentially interfere with CD40 based leukaemia therapies.

An ELISA was developed and sCD40 levels measured in normal donors and leukaemia patients for whom we had survival data. An analysis of the clinical significance of sCD40 levels was then performed.

Levels of sCD40 were significantly elevated in a proportion of leukemia patients. In both Myeloma and Acute Myeloid Leukaemia (AML) patients, elevated sCD40 levels were a significant independent predictor of poor survival.

The circulating levels of sCD40 in AML and myeloma patients provide a new prognostic marker in Myeloma and AML. The presence of elevated sCD40 levels may also reduce the effectiveness of CD40 based therapies.

**Immunocytochemistry standardisation, automation and image analysis**
***M Ibrahim. UK NEQAS, London, UK***

Immunocytochemistry is still evolving tremendously, with an unpredictable number of novel markers released on the market over the next coming years, which will have further impact on patient care. Although paraffin-wax sections are now routinely used, standardised protocols largely remain 'standard' for a specific antigen. Furthermore, the increase in laboratory workloads and technology has fuelled the needs for automated slide processing systems. However, automated systems will need further changes in already established 'manual' protocols, which will require further internal and external quality control.

Image analysis is another realm in immunohistochemistry, which is beginning to advance from the field of research to the clinical laboratory. Advantages include removing of subjective scoring criteria and the possibility to archive digital slides. The UK National External Quality Scheme for Immunohistochemistry (UK NEQAS-ICC) has a great advantage to observe, first hand, the changes which are occurring in scheme members from 38 international laboratories. With respect to image analysis, the UK NEQAS-ICC envisages an opportunity for image analysis to 'assist' in the assessment of participants' slides. With over 5000 participant slides being manually assessed we also have the opportunity to scrutinise all aspects of image analysis, which may soon be an additional tool available in clinical diagnosis. Standardisation, automation and image analysis, once combined, may have the potential to assist pathologists and biomedical scientists in more accurately predicting clinical response to therapy. tumours.

**Rapid detection of MRSA**
***T Anderson. Canterbury Health Laboratories, Christchurch***

*Staphylococcus aureus* is a major pathogen responsible for nosocomial and community-acquired infections. Staphylococcus aureus resistance

to penicillin soon developed after its introduction and within the last 20 years has seen the evolution and globalisation of the methicillin-resistant Staphylococcus aureus. This organism has become a major financial burden to health care institutions and requires effective infection control strategies for its control. Microbiology isolation procedures need to be rapid, sensitive and specific and molecular techniques such as PCR fulfill this requirement.

### Inborn errors of metabolism

*C Leaver. Canterbury Health Laboratories, Christchurch*

Inborn errors of metabolism are inherited genetic disorders in which a protein such as an enzyme, a receptor, or a carrier protein does not function properly. They are individually rare but collectively numerous, affecting approximately 1 in 500 new-borns. These conditions (over 400 in all) can present in many different ways at any age, although they are most common in infancy and childhood. Pathophysiologically the inborn errors can be divided into 3 diagnostically useful groups:

1. Disorders that give rise to intoxication from accumulation of toxic compounds proximal to the metabolic block. eg aminoacidopathies (phenylketonuria, homocystinuria etc); most organic acidaemias (glutaric aciduria Type1, isovaleric acidurias etc), urea cycle defects, and sugar intolerances (galactosaemia, hereditary fructose intolerance). Symptoms may be acute (vomiting, lethargy, coma, liver failure) or chronic (progressive developmental delay, cardiomyopathy).
2. Disorders involving energy production or utilisation (glycogenosis, gluconeogenesis, congenital lactic acidaemias, fatty acid oxidation defects, respiratory-chain disorders). Symptoms include failure to thrive, hypoglycaemia, metabolic acidosis, hypotonia, cardiomyopathy, sudden infant death syndrome.
3. Diseases that disturb the synthesis or catabolism of complex molecules. Symptoms are permanent, progressive and not related to food intake, e.g. mucopolysaccharides, peroxisomal/ lysosomal disorders, congenital defects of glycosylation, etc.

### Audit of the audit

*D H Roche. Southern Community Laboratories*

The Cervical Cancer Audit [CCA] reviewed two groups of slides: negative control slides, and slides reported as negative in women who subsequently developed cervical cancer. Some of the slides from each group were upgraded to high grade. At least 2 of the upgrades of the negative controls have been confirmed as benign by follow up and colposcopy. Six of the upgrades from the women with cancer were reviewed regularly and reliably as negative using a method of multiple slide blinded review. A stricter false negative rate for HSIL (5%) than the NCSP quality standard (20%) was used. These findings limit the conclusions that can be drawn from the results slide review component of the CCA. The results do not provide a guide to what abnormalities a reasonable and prudent laboratory should have detected. Nor do they provide useful information to women who worry about the quality of the reporting of their cervical smears.

### Hodgkins lymphoma: a diagnostic dilemma

*K V Pillay. Diagnostic Medlab Ltd*

The cytologic features of Hodgkins Lymphoma (HL) ,has been well documented, HL still presents as a diagnostic challenge to most cytology screeners. This presentation highlights the cytologic features that aid in making a correct diagnosis, also highlighting the factors that contribute to a false negative diagnosis of HL.

Cases of HL, evaluated by fine needle aspiration (FNA), were identified between January 2002 -June 2005. All were lymph node samples. Histological correlation was available for these cases. Based on the original cytologic diagnosis, 10 cases were positively reported as HL, 14 cases were suggestive for HL on cytology, i.e. a suspicious or atypical diagnosis (subsequent biopsy proven cases on histology), three false negative cases (sampling error and fibrosis of the lymph node), and two cases were reported as granulomatous lymphadenitis, of which one was reclassified as a HL with granulomatous lymphadenitis.

The diagnostic dilemma of HL in both the suspicious and false-negative cytologic cases were the absence of classic Reed-Sternberg cells. Contributing factors for these equivocal cytologic diagnosis include the following: lack of a reactive background; lack of neoplastic cells; reactive inflammatory cells .eg. neutrophils obscuring neoplastic cells; partial involvement of the LN by HL; fibrosis of the lymph node; and inadequate sampling of the lymph node. Histologic assessment with immunophenotyping will assist in improving the cytologic diagnosis of HL.

### The Christchurch tissue bank: a biorepository for cancer research

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Breakthroughs in the understanding of cancer biology, prognostic indicators and development of novel treatments are increasingly dependent on accessing human cancer tissues with their associated clinicopathological data. The Christchurch Tissue Bank (CTB) has been established to meet this need, by developing a central repository of consented cancer tissues for genomic and proteomic studies. It is a collaboration involving staff from the Christchurch School of Medicine and the Canterbury District Health Board. The CTB operates to international biorepository standards but must also comply with New Zealand's ethical, legal and cultural requirements. Patients attending the central pre-admission clinic, Christchurch Hospital, before cancer surgery are informed of the CTB, and given the opportunity to gift tissue, excess to diagnosis, to assist future cancer research. This has enabled the storage of serum, plasma, DNA, fresh frozen tissue, OCT compound embedded blocks, slide preparations, paraffin blocks and tissue microarrays, with their associated clinicopathological data from over 18 different cancer tissue types involving over 2000 patients.

### Myeloproliferative disorders

*P Ganly. Canterbury Health Laboratories, Christchurch*

Although the WHO has classified many entities under the heading chronic myeloproliferative diseases or myelodysplastic/myeloproliferative diseases, this session will focus on the related disorders polycythaemia vera and essential thrombocythaemia. With the exception of chronic myelogenous leukaemia (also classified under the myeloproliferative heading) these two disorders are the most clearly defined. There has been controversy about which diagnostic features are required in order to make either of these diagnoses unequivocally - there is still a need in some patients for watchful waiting with an open mind before excluding myeloproliferative disease as a cause of elevated hamatocrit or platelets. It has very recently emerged that most patients with polycythaemia vera and a significant minority of those with essential thrombocythaemia have a recurring mutation in the tyrosine kinase

gene JAK2. This has the potential to alter the diagnostic algorithm, may impact on prognosis and sheds light on pathophysiology in these diseases. There has also been controversy about whether in some patients treatment is necessary and if so with what. Recent studies have defined more clearly the natural history of these disorders using various treatment programs and it is now possible to outline an evidence based approach for most patients.

**Leucodepletion**
***P Flanagan. New Zealand Blood Service, Auckland***

The NZBS introduced universal leucodepletion during 2000. This was part of a package of measures aimed at reducing the risk of transmission of variant CJD by transfusion. This was consistent with the approach utilised in the United Kingdom. The beneficial effect of leucodepletion in the context of vCJD remains controversial. The decision to introduce universal leucodepletion occurred at an early stage in the development of a national blood service. It enabled the development of a standard range of blood components across the country linked to the implementation of standardised technology at the four NZBS manufacturing sites. The flow on effects of this with respect to ease of transfer of components between sites greatly facilitated the introduction of a truly national approach to management of stock. The technology itself has proven to be very robust. Filter failure is an uncommon event. Statistical process control methods have demonstrated a high level of confidence that individual units will meet the required level of less than $5 \times 10^6$ white cells per unit. Universal leucodepletion has been increasingly adopted by Blood Services internationally over the last several years. Debate continues on the immunomodulatory benefits associated with leucodepletion. It is now recognised to be as effective as CMV antibody screening in the prevention of CMV transmission by transfusion. The demand for CMV antibody screening however continues, this reflecting the innate conservatism of the blood transfusion environment.

**Pharmacogenetics and drug metabolism: is it relevant to clinical practice?**
***S J Gardiner, E J Begg. Christchurch School of Medicine, Christchurch***

Interindividual variability in the expression of drug metabolising enzymes (DMEs), transporters and receptor proteins has a major impact on drug response, such that unexpected toxicity or inefficacy may result from 'standard' doses. There has been much hype about the clinical potential for pharmacogenetic profiling over recent years, especially in relation to DMEs. However, clinical uptake remains low. A recent Australasian survey (1) demonstrated that the only pharmacogenetic tests for DMEs performed with any regularity for patient benefit were thiopurine methyltransferase (TPMT) and butyrylcholinesterase. In 2003, 2,000 - 2,500 tests were undertaken for each of these enzymes, with phenotyping favoured over genotyping. Prospective testing for TPMT is 'cost-effective' and identifies the 0.3 - 0.6% of the population with enzyme deficiency who will almost certainly develop severe myelosuppression with usual azathioprine or 6-mercaptopurine doses. Testing for butyrylcholinesterase is established, either retrospectively after prolonged apnoea with suxamethonium or mivacurium, or prospectively in susceptible families. While a strong case for testing for these two enzymes can be made, they are relevant to only four drugs. In contrast, the key polymorphic cytochrome P450 enzymes (CYP2C9, 2C19 and 2D6) are involved in the metabolism of more than 25% of all drugs but there is insufficient evidence to support testing for these enzymes routinely in clinical practice. The low use of pharmacogenetic tests for DMEs may reflect factors such as the inadequacies of available research data, lack of clinical relevance and, in a few cases, a slow transition from a strong evidence basis to the clinical setting.

1. Gardiner SJ, Begg EJ. *Pharmacogenet Genomics*. 2005; 15: 365-9.

**Eosinophilias**
***P Ganly. Canterbury Health Laboratories, Christchurch***

The occurrence of eosinophilia (abnormally increased eosinophils to $0.7 \times 10^9$/l or more) is commonly identified with automated blood count analysers. Numerous conditions are associated with or cause eosinophilia - to make any sense of these in an individual patient it is most practicable to concentrate on those with the more significantly elevated eosinophil counts. Less than 1% of blood counts in a general hospital laboratory have moderate eosinophilia $> 1.5 \times 10^9$/l and only a handful of these have severe eosinophilia $> 5 \times 10^9$/l. Production of eosinophils is supported by a variety of cytokines, including interleukin 5, and in some diseases eosinophilia is due to a particular type of T-cell response to antigens in allergic or parasitic diseases. In others, eosinophilia may be part of a malignant process, either non haematological or haematological. Some cases of idiopathic hypereosinophilia can now be reclassified as myeloproliferative disease based on recent evidence of acquired clonal change. Irrespective of the cause of eosinophilia, these cells with cytotoxic properties may cause tissue damage. Treatment is directed at the underlying cause and also at preventing or reducing the downstream effects of eosinophilia.

**The glorious liver sieve**
***R Fraser. Christchurch School of Medicine & Health Sciences, Christchurch***

The one billion 1mm long hepatic sinusoids, all in parallel, within the one million primary lobules of the liver, and lined by a thin fenestrated endothelium separating the blood from the space of Disse and hepatyocytes, constitute the 'liver sieve.' Each sinusoid contains blood which is sluggish in movement, with cramped blood cells just able to roll or push along its length, massaging the gossamer-like endothelium with its myriad of 100nm fenestrae. These facilitate the exchange of plasma, proteins, most lipoproteins, electrolytes and nutrients between the external environment, from the gut (portal), internal environment (hepatic artery) and that synthesised by the hepatocytes. We were the first to demonstrate the separation by the sieve of the largest of lipoproteins, the chylomicrons with their dietary fat, from their smaller remnants, depleted of their fat but rich in dietary cholestrol and fat soluble vitamins. The liver sieve has since been shown to modulate exogenous and endogenous metabolism, hormone regulation and immune rejection and tolerance. Aberrations of the sieve influence many diseases both within the liver, such as steatosis and cirrhosis, and outside the liver such as atherosclerosis, osteoporosis, perhaps cancer and autoimmunity, ageing (with Le Couteur's Sydney team) dementias and reaction to detergents and surfactants.

**Rapid influenza testing**
***L C Jennings, Virginia Wells, M Smit, D R Murdoch. Canterbury Health Laboratories and Christchurch School of Medicine & Health Sciences, Christchurch***

Influenza outbreaks and epidemics pose on-going risks to community human public health. The confirmation of influenza activity in many countries relies on active surveillance networks, which can provide

"early warning"information to clinicians and assist the accuracy of the clinical diagnosis of influenza. The increased awareness of the severity of influenza and of possible control measures, including specific antivirals, has encouraged the development of rapid influenza tests, sometimes called "near-patient" or "point-of-care" tests, for the confirmation of influenza in a variety of clinical settings. Rapid testing, especially at the beginning of an influenza season or outbreak, may influence clinical decisions. However, rapid tests differ in their complexity and clinical accuracy, and knowledge of their limitations is essential prior to their use.

**Inquilinus limosus: you mean you've never heard of it?**
***E Keith. Canterbury Health Laboratories, Christchurch***

Burkholderia cepacia is an opportunistic pathogen that causes pulmonary infections in individuals with cystic fibrosis and chronic granulomatous disease. Due to resistance to most antibiotics treatment is difficult and the prognosis is poor. Management is therefore based on prevention by strict infection control measures. As this can have a huge impact on patients and their families, accurate identification is essential. Although first recognized in 1950, the taxonomy of Burkholderia species is constantly changing. Currently there are 22 recognised species with 8 closely related genomic species or genomovars making up the *B. cepacia* complex. Using selective media, phenotypic and biochemical methods available to routine laboratories, members of the *B. cepacia* complex are difficult to distinguish from each other and other closely related species. Studies using molecular methods have shown that a number of species, including recently recognized species such as *Inqilinus limosus*, are commomly misidentified as *B.cepacia*. As this can have serious consequences it is therefore recommended that identification should be based on molecular methods.

**OH & S prescreening and needlesticks**
***C Wong. Department of Forensic Medicine, Glebe, Australia***

In NSW, Forensic Medicine centres and hospital mortuaries undertake both non-coronial and coronial autopsies. Each area has obligation to NSW occupational health and safety legislation. Autopsy procedures concerning HIV and HCV positive bodies vary across the state. These policies and procedures may affect family wishes in relation to body viewing prior to autopsy or to have body reconstructed before burial or cremation. Protection of mortuary personnel against exposure to these blood-borne pathogens has becoming an important OH&S issue. This project aimed at identifying appropriate risk control strategies to minimise the risk of exposure of blood-borne diseases associated with the reconstruction of bodies following autopsy. Its findings gave an overview of the relevant research based on evidence to pathogen exposure and nature of risk, a summary of the current work practices in NSW and an account of factors known to be linked with elevated levels of risk.

**Implentation of multi-slice technology in a forensic mortuary**
***S M Collett. Victorian Institute of Forensic Medicine, Melbourne, Australia***

In May 2005, a 16-slice Computed Tomography (CT) Scanner was installed in the mortuary at the Victorian Institute of Forensic Medicine (VIFM). This is the first forensic mortuary in Australia to install a CT scanner, and the first mortuary in the world to install a 16-slice CT scanner. The scanner provides us with a unique opportunity to view deceased persons without extensive dissection. The main purpose of this new imaging modality is to assist in forensic pathology examinations, and to facilitate and enhance the VIFM's ability to quickly identify large numbers of deceased persons in the event of a mass fatality incident. Forensic technical staff and anthropological specialists underwent training in the various aspects of the scanner operation and image acquisition, and have obtained operating licenses. Forensic pathology staff have received training in the viewing and reconstruction of images. It is envisaged that from the 1st of August 2005, all deceased persons who are admitted to the VIFM will receive a whole body scan, or 'head to toe' scan, as part of the admission procedures. Dedicated re-scanning of specific anatomical areas for more detailed studies will also be performed as each case dictates. By using the CT technology as a tool to assist the forensic autopsy, the process first needs to be validated so that it can withstand scrutiny by defence council in the courts. The development of protocols concerning diagnostic and research applications will also be necessary so that the maximum benefit can be gained from this exciting innovation.

**Sensitivity of Pap Smear for Detection of HSIL**
***D H Roche. Southern Community Laboratories***

**Aim:** All cervical histology was correlated with concurrent or previous cytology within the preceding 18 months in an effort to determine the sensitivity of the conventional Pap smear.

**Methods:** All cases in which histology and cytology were discrepant by >1 degree of SIL (Normal, ASC-US/LSIL, ASC-H/HSIL) were reviewed retrospectively to determine the cause of the discrepancy and in particular whether abnormal cells had been missed on the Pap smear.

**Results:** Of 388 cases of HSIL, 86% had previous smears which had been diagnosed as abnormal (ASC-US and up). Fifty-five % of these abnormal smears were called HSIL. The specific sensitivity for HSIL = 55% and the complete sensitivity = 86%. The false negative rate of the test for HSIL = 14% (ASC-US and up). Nine of the 45 negative smears had abnormal cells (ASC-US and up) present in retrospect by biased unlimited review. Recognition of these cells could potentially reduce the false negative rate down to 11%.

**Conclusions:** The sensitivity of the Pap smear for detection of HSIL is estimated at 86% although many of the abnormal smears demonstrated a lower grade of SIL. The specificity (PPV for HSIL) is 87%. A small number of false negative cases were identified which could potentially improve the sensitivity to 89%.

**Medical problems at high altitude**
***D R Murdoch. Canterbury Health Laboratories, Christchurch***

High altitude illness is a risk to people travelling to >2500 m and is a collective term for three syndromes: acute mountain sickness (AMS), high-altitude cerebral oedema (HACE) and high-altitude pulmonary oedema (HAPE). Despite intensive investigation, the precise causes of high-altitude illness are uncertain, and it is difficult to predict who is likely to be affected. Gradual ascent is the best means of preventing high-altitude illness, although some drugs have proven prophylactic value. Other medical problems at high altitude include diseases associated with sun and cold exposure. In addition, some medical problems are potentially aggravated by high-altitude exposure.

**Operation Phuket: disaster victim identification in Thailand following the Boxing Day tsunami**
***S Stables. Auckland Hospital, Auckland***

On Dec 26th, 2004 an earthquake centred in the Sundra trench, just

off Indonesia triggered a tsunami which caused massive destruction and loss of life in the countries rimming the Indian Ocean. One of the worst affected regions was Khao Lak in the south of Thailand, a popular destination for foreign tourists. The first deployment of NZ personnel to Thailand was the NZ Police DVI (Disaster Victim Identification) Squad of which the presenter is a member. The squad arrived in Phuket, Thailand on New Year's Eve and spent three weeks working there. The presentation will focus on the work of the NZ Police and forensic specialists in identifying the deceased in the Khao Lak region. The identification of victims of a mass disaster must be methodical and exact, can not be compromised due to public pressure, and due to the nature of the disaster, may take some time before the deceased can be released to the family.

**Evaluation of a post thaw viable CFU-GM assay and association with time to haemopoietic stem cell recovery**

***S Carnoutsos[1], V Buchan[1], H Patel[2], J Sanders[3]. [1]Canterbury Health Laboratories, [2]NZBS Southern and [3]Clinical Haematology Unit, CDHB, Christchurch***

In March 2004 the Haemopoietic Stem Cell Processing Laboratory at Canterbury Health Laboratories adopted a viable colony forming unit - granulocyte/macrophage (CFU-GM) assay based on experience gained from working in an Australian facility and following in-house comparison studies. As a result of the work the threshold for the number of CFU-GM required to satisfy local engraftment criteria was lowered from 5.0 x $10^4$/kg to 3.0 x $10^3$/kg and the requirement for pre-cryopreservation testing was eliminated.

**Homozygous Haemoglobin E and G6PD deficiency - a case study**

***J M Duncan. Canterbury Health Laboratories, Christchurch***

A routine request for the investigation of a possible haemoglobinopathy on a South East Asian (SEA) child provided a surprise diagnosis. Standard haemoglobinopathy testing concluded the child was homozygous for Hb E, a common chain haemoglobin variant prevalent in SEA. As part of the screen a blood film was examined which showed not only the target cells usually associated with Hb E but also irregularly contracted cells suggestive of oxidant stress. Further testing confirmed the child was deficient for glucose-6-phosphate-dehydrogenase (G6PD), a red cell enzyme required for maintenance of the hexose monophosphate pathway. G6PD deficiency is frequently encountered throughout Asia. The inheritance of both these genetic disorders raises the question of a possible increase in the severity of haemolysis associated with oxidative stress compared to that of G6PD deficiency alone.

**Activated seven lupus anticoagulant - ASLA. A new diagnostic assay**

***D Patterson, K Allan, A Ruddenklau, M Smith. Canterbury Health Laboratories, Christchurch***

The interpretation of assays for the detection of a Lupus Anticoagulant (LA) can be difficult. That no one method is predictably positive for LA reflects the heterogeneity of these antiphospholipid antibodies. The combination of the Dilute Russel Viper Venom Time (DRVVT) and Kaolin Clotting Time (KCT) methods will detect 80-85% of patients with LA. A supplementary method is required to clarify equivocal results and to increase sensitivity of LA detection. We have developed an additional assay for LA using recombinant activated factor VII, the ASLA assay. It is an extrinsic based clotting assay utilising a platelet neutralisation step as the confirmatory test. Ratios of patient to normal control clotting times are analysed with abnormal ratios undergoing the platelet neutralisation step. The ASLA is used to evaluate samples previously tested for LA that gave equivocal results. Results demonstrate a high detection rate with 12 of 18 (67%) KCT positive, and 14 of 15 (93%) known LA, testing positive using the ALSA assay. We propose that the ASLA assay can be used as a supplementary test for the detection of LA where conventional methods have shown equivocal results.

**IFAT serology for Legionellosis. Significant variables affecting interlab comparability**

***A L R Southern. ESR Kenepuru, Porirua***

A major function of the Legionella Reference Laboratory is to confirm preliminary serological findings and identify the causative agents of legionellosis using a wider range of methodologies than those available to most diagnostic laboratories. Results reported by ESR may vary from those obtained by the referring laboratory. This may in part be the result of different methodologies but may also reflect other variables:

- Commercial kits may contain limited species and strains ( eg single strain *L. pneumophila* sg 1) and species which cross react. ESR uses four strains of *L. pneumophila* sg 1, two of *L.pneumophila* sg 4 and a wider range of non pneumophila antigens than those included in kits currently available in NZ.
- Use of kits with conjugates other than those recommended by the manufacturer may increase titres.
- Use of any conjugate at concentrations greater than optimum determined by titration will give false high titres.
- Test sensitivity is improved by using a broad spectrum FITC conjugate.
- The IFAT method uses CBR to absorb out campylobacter antibodies which can cause false positives. This may reduce observed titres by one doubling dilution. False positive titres in cases of campylobacter gastroenteritis become negative.
- Cross reactions can occur with other Legionella species and non legionella organisms other than campylobacter. Improved understanding of the variables affecting the Legionella IFA is necessary to better correlate interlab reports and interpret results for end users.

**Antibiotic susceptibility of extended-spectrum â-lactamase producing Enterobacteriaceae**

***R Woodhouse, H Heffernan. Institute of Environmental Science and Research Ltd***

**Purpose:** Extended-spectrum â-lactamase (ESBL)-producing organisms are being isolated with increasing frequency in New Zealand (NZ), and standardly confer resistance to 3rd and 4th-generation cephalosporins and monobactams. Overseas studies often report ESBL-producing organisms to be resistant to several additional classes of antibiotics, especially fluoroquinolones and aminoglycosides. Little has been documented about antimicrobial resistance among ESBL-producing organisms in NZ. We therefore determined the susceptibility of ESBL-producing Enterobacteriaceae, recently isolated in NZ, to a range of antibiotics.

**Methods:** As part of a survey of methods to detect ESBLs, we tested the susceptibility of a representative sample of 146 isolates of ESBL-producing Enterobacteriaceae to amikacin, ciprofloxacin, co-amoxiclav, co-trimoxazole, gentamicin, meropenem, nitrofurantoin, tetracycline, tobramycin and trimethoprim by the CLSI/NCCLS agar

dilution method.

**Results:** 83% of *Escherichia coli*, 55% of *Klebsiella*, and 90% of other Enterobacteriaceae species were multiresistant to 3 or more classes of antibiotics in addition to cephalosporins and monobactams. The dominant pattern displayed by 48% of *E. coli* was aminoglycoside + ciprofloxacin + folate pathway inhibitor + tetracycline ± co-amoxiclav resistance. No multiresistant pattern was dominant among *Klebsiella*. The most common pattern among other Enterobacteriaceae was co-amoxiclav + aminoglycoside + folate pathway inhibitor + tetracycline resistance. All isolates were susceptible to meropenem.

**Conclusions:** ESBL-producing Enterobacteriaceae in NZ have high rates of resistance to antibiotics in addition to cephalosporins and monobactams, and are frequently multiresistant.

### Comparison of plastic and glass: gel and non-gel blood collection tubes for a variety of analytes

***R Van Der Hilst, J Chew. Southern Community Laboratories, Christchurch***

**Purpose:** Our laboratory currently uses glass tubes (serum) mostly for laboratory blood analysis. Due to safety reasons, we are currently considering the change from using glass tubes to plastic tubes. Because of possible interference caused by adsorption of components to plastic wall tubes, we aimed to assess any differences for a number of analytes across a variety of tube types.

**Methods:** A total of 38 volunteers participated in this study. Blood was taken from each volunteer into four different Becton Dickinson blood collection tubes - glass plain, plastic plain, glass SST and plastic SST. All tubes were centrifuged at 3000 rpm for 10 minutes before analysis. Tests in this study included prostate specific antigen (PSA) and hepatitis B surface antigen which were analysed on the Axsym (Abbott); calcium, magnesium, phosphate, albumin, lipids, iron, iron binding capacity and ferritin which were tested on the Hitachi 917 (Roche); thyroid functions tests, testosterone, fertility hormone tests, folate and B12 which were analysed on the Advia Centaur (Bayer).

**Results:** Paired t-test and Passing-Bablock agreement analysis was used to investigate the differences between the tube types. For any comparison, the mean difference (and its 95% confidence interval) was expressed as a percentage of the mean of the analytes on that pair of tubes.

**Conclusions:** Statistically, we found analytes that were mostly affected by using different tube types were ferritin, PSA, folate, B12 and follicle stimulating hormone. The biggest differences were noted in B12, Folate and Ferritin.

### Low HDL: More than just a risk factor of coronary heart disease

***M Reed, M Boyer. SCL, Christchurch***

HDL cholesterol levels are routinely used in risk assessment of Coronary Heart Disease (CHD). We present two case studies, displaying unusual and anomalous HDL cholesterol results. The first case is that of a 69-year-old male, who presented for routine lipid screening, and demonstrated a very low HDL cholesterol level. This anomalous result lead to the addition of liver functions, and when the total protein was found to be elevated, serum protein electrophoresis and Bence Jones Protein were also added. Immunofixation was used to confirm the presence of an IgG kappa paraprotein. Investigation of the initial anomalous result has allowed the discovery of a previously undiagnosed case of myeloma. The manufacturer of our HDL cholesterol reagent states that *"In rare cases, gammopathy, in particular type IgM (Waldenstrom's macroglobulinaemia), may cause unreliable results"*. However, in this case and in our second case, the paraproteins are both IgG kappa paraproteins. These highlight the potential interference in our HDL cholesterol method, and demonstrates the importance of identifying the reason for anomalous results.

### Thrombotic thrombocytopenic purpura and ADAMTS-13

***A Wong. LabPlus Auckland Hospital, Auckland***

Thrombotic thrombocytopenic purpura (TTP) is a rare disease that can be either congenital or acquired. The reduced activity of von Willebrand factor (VWF) degradation has been related to TTP. ADAMTS-13 is a VWF-cleaving protease. Levy et al. (2001) have found that a deficiency of this gene is the molecular mechanism responsible for TTP. Patients with congenital TTP inherit either homozygous or compound heterozygous mutations of the ADAMTS-13 gene. They quite often suffer from recurrent episodes and are found to have more than one mutation in the gene. The acquired form of TTP is caused by autoantibodies acting against VWF cleaving protease. The autoantibodies against ADAMTS-13 is responsible for the decreased levels of VWF cleaving protease in these patients.

### The epidemiology of meningococcal disease in New Zealand, 2004

***R McDowell, H Davies, L Lopez, D Kay. Institute of Environmental Science and Research, Porirua***

**Purpose:** To provide summarised surveillance data for meningococcal disease in New Zealand in 2004.

**Methods:** Data on each case of meningococcal disease are collated with the strain identity of patient samples. Isolates are serotyped, and PCR and sequencing are used to characterise DNA from clinical samples and untypable isolates.

**Results:** In 2004, 342 cases of meningococcal disease were notified, a rate of 9.2 per 100 000 population. 273 (79.8%) of these cases were laboratory confirmed. There was a marked geographic variation in the rates of meningococcal disease. The highest age-specific rates were in those aged less than one year (84.2 per 100 000 population) and 1-4 years (44.4 per 100 000 population). The age-standardised rates for Maori and Pacific Peoples were at 1.7 and 3.2 times the European rates. Of the 252 cases where samples could be characterised, the epidemic strain accounted for 73.0% (184/252). Group C accounted for 20 cases (7.9%). There were 8 deaths in 2004, a case-fatality rate of 2.3%, the lowest to date throughout the course of the epidemic.

**Conclusions:** This disease continues to be one of the most important notifiable diseases in New Zealand. The total number of cases reported from 1991 through 2004 is 5635, which is an excess of 5582 cases had the 1990 case numbers persisted. Accurate disease surveillance data including laboratory confirmation of disease will be vital for evaluation of the effectiveness of the strain-specific vaccine, MeNZB(™).

### Rapid influenza testing - the impact on patient management

***V C Wells, M Smit. Virology. Canterbury Health Laboratories, Christchurch***

Peak influenza virus activity occurs during winter months and increases pressure on healthcare resources annually. To assist with patient management during periods of influenza activity, Canterbury Health Laboratories provides a 24 hour/ 7 day rapid influenza testing service to the emergency departments at Christchurch Hospital. This service is activated once influenza surveillance indicates increased

circulation of influenza A or B viruses in the community. The impact of rapid influenza testing on patient management was determined by analysing diagnostic and related patient data from 2003. Adult patients with positive rapid influenza tests were less likely to be admitted to hospital and had fewer additional pathology tests requested. This finding was more pronounced in patients less than 60 years of age. Children were less likely to be admitted or prescribed antibiotics. Rapid influenza testing may have contributed to an observed decrease in the rate of confirmed nosocomial influenza infection - 6% in 2003 compared to 12% in 2002. Our findings suggest rapid influenza testing aids patient management and infection control strategies, reduces hospital admissions and rationalises pathology test requests.

**A histological approach to dental research**
***Sarah McKenzie. Wellington School of Medicine, Wellington***

Histology is now one of a suite of techniques used by the Dental Research Group in the Wellington School of Medicine to study plaque. Recent histology work has involved a double embedding method using agarose and paraffin wax to allow researchers to study the 3D structure of dental plaque. The plaque is grown in a unique laboratory model known as the 'ultiplaque artificial mouth' culture system. This system allows large biofilms, or plaques to be grown that can be sampled for TEM, SEM, histology and fluorescence studies. Due to the friable nature of the plaque, the samples are first embedded in agarose which allows them to be routinely processed, wax embedded and sectioned. The main advantage of using agarose is that it maintains the native structure of the plaque and prevents loss of sample during processing. The use of histology stains such as Toluidine Blue and Gram stain have enabled researchers to see specific differences in plaque architecture and describe its heterogenous structure. Histology alongside electron microscopy will aid the development of effective oral therapies and the study of the pathogenesis of oral disease through the study of plaque composition and structure.

**Acquired Glannzmann's thrombasthenia: a case report**
***C McLintock, S Jones. Diagnostic Medlab, Auckland***

Acquired Glannzmann's thrombasthenia is a rare haemorrhagic disorder due to the development of an auto-antibody to the platelet receptor GPIIb/IIIa that leads to defective platelet aggregation. We report a case of possible acquired Glannzmann's thrombasthenia detected in a woman who presented with a history of recent onset of severe menorrhagia that required blood transfusion. Prior to the development of severe menorrhagia our patient had no bleeding history and had one child born 4 years previously by caesarian section with no bleeding complications. Investigations - bleeding time >20 minutes. Normal APTT, PR, fibrinogen and von Willebrand factor parameters. Platelet aggregation studies - classical Glannzmann's phenotype: absent aggregation to ADP, adrenaline and collagen, normal response to ristocetin. Immunoenzyme staining of platelets: positive for GpIIb, GpIIIa and GpIb alpha. Serum platelet antibodies and platelet associated antibodies demonstrated. MAIPA studies antibodies were detected to anti-GP IIb/IIIa and anti-GP Ia/IIa glycoproteins. The clinical history and laboratory findings support a diagnosis of acquired rather than inherited Glannzman's thrombasthenia. Currently this woman's menorrhagia is controlled with the oral contraceptive pill and she has no other significant bleeding symptoms. Our patient would like to have another child but with acquired Glannzman's thrombasthenia would be at risk of severe bleeding in the peri-delivery period. In other case reports, immunosuppressive therapy has been successful in treating bleeding symptoms and we are considering this as a possible therapeutic option.

**Renal stone analysis by X-ray diffraction - a New Zealand experience**
***S H Woods, P M George. Canterbury Health Laboratories, Christchurch***

Traditional wet chemistry techniques for renal stone analysis use a series of spot tests to determine the composition of the stone. This is labour intensive, operator dependant and may give erroneous results. In 1998 Canterbury Health Laboratories changed from wet chemistry to X-ray diffraction for renal stone identification. X-ray diffraction is a quick and simple, non-destructive procedure that requires a small quantity of sample material. In the X-ray diffraction method, compounds that possess specific crystalline structures are identified. X-ray diffraction can identify major and minor constituents of composite stones and provides the hydration status of the constituents. In our experience the change from wet chemistry to X-ray diffraction has achieved short turnaround times and improved accuracy as shown by external quality assurance. We have identified a wide range of minerals in gallstones, bladder stones, renal stones, salivary stones and other crystalline deposits in urine.

**D84E, a novel Ferroportin linked to iron overload**
***T A Walmsley, J Harraway. Canterbury Health Laboratories, Christchurch***

Primary haemochromatosis is an autosomal recessive disorder in which iron is deposited in the liver, pancreas, and other organs, leading to cirrhosis of the liver, diabetes and cardiovascular disease. In Caucasians this is usually due to homozygous mutation of the HFE gene C282Y, but some cases are compound heterozygotes for C282Y and the H63D mutation, or have other mutations. There are now six genes known to cause clinically significant iron overload. Raised serum iron saturation and ferritin suggest a diagnosis of haemochromatosis but HFE genotyping is usually done to support the diagnosis. The hepatic iron index is a reliable method for establishing iron overload and, in a patient without any cause of secondary iron overload, an index of >2.0 μmol/g/year is essentially diagnostic of haemochromatosis. Here we describe 67 year-old male who presented after a diagnosis of haemochromatosis was made in his brother. He had a ferritin of 3582 μg/l and iron saturation of 90% with iron overload confirmed by a raised hepatic iron index (5.0 μmol/g/year). HFE genotyping revealed that he was heterozygous for the H63D mutation and, subsequently, that his brother had neither of the common HFE mutations. This excluded a recessive HFE linked disorder and, since the proband's daughter was also reported as having iron overload, suggested a dominant inheritance pattern. DNA sequence analysis of the ferroportin gene revealed a novel mutation (D84E). This mutation segregated with the iron overload, is located in an extracellular loop between transmembrane segments 1 and 2, where other pathogenic mutations have previously been identified and Asp84 is highly conserved across known species. In combination this data suggests that the D84E ferroportin mutation causes iron overload.

**Validation of 1H NMR spectroscopy as an analytical tool for methylamine metabolites in urine**
***M B Lee, M K Storer . Canterbury Health Laboratories, Christchurch***

**Background:** Methylamines have many metabolic roles and there is an increasing demand for their measurement. Glycine betaine is an important osmolyte, and a reservoir for methyl groups. Proline betaine and trigonelline are important dietary betaines. Dietary trimethylamine is normally converted to trimethylamine oxide but in fish odor syndrome is excreted as TMA. These compounds are all suitable for quantification by 1H NMR spectroscopy as they all have methyl protons.

**Methods:** Urine samples are acidified and 1H NMR spectra are obtained using presaturation for water suppression. Peak integrals or heights are compared to an internal standard of acetonitrile.

**Results:** Inter and intra-assay CV's were < 5% for TMAO and creatinine, and < 10% for the other analytes. Responses were linear from 50 M to 1000 M for all metabolites, and recoveries were 97%. Limits of detection using NMR are slightly higher than alternative HPLC assays (15 ?C 25 M). However, sensitivity is adequate for the detection of raised levels in urine, and sample analysis was complete in less than five minutes.

**Conclusions:** 1H NMR spectroscopy is a convenient, rapid and economical option for the determination of betaines and related compounds in urine in a single analysis.

**An unusual structurally rearranged Y chromosome associated with azoospermia**

***E Moore, S Thomas, S Baker, J Crolla, J Barber. Wessex Regional Genetics Laboratory, Salisbury, UK***

A 36 year old male was referred for azoospermia and a possible diagnosis of Klinefelter syndrome. On G-banded analysis, his Y chromosome appeared to be monocentric with two copies of the short arm and little, if any, long arm material. Fluorescence in situ hybridization (FISH) confirmed the presence of two copies of SRY and one centromeric signal but molecular anaysis showed that AZFa and AZFb were still present. AZFc was deleted consistent with azoospermia. Further FISH with a panel of Yp and Yq YACs showed that the Yq breakpoint was between 700C1 and 539D10 within Yq11.23 and the Yp breakpoint was within Y190 (Yp11.2) which showed a split signal. In addition, the Xp/Yp subtelomeric signal on the der(Y) short arm had increased signal intensity compared with the other Yp and Xp subtelomeric signals. The karyotype was therefore 46,Xder(Y)(pter::pter-q11.23::p11.2-pter). The complex nature of this der(Y) would not have been suspected without the additional molecular analyses and suggests the existence of a novel sub-set of non-fluorescent Y chromosomes associated with azoospermia in man.

**Rapid in SITU harvesting and cytogenetic analysis of amniotic fluid samples: the validation of a new service for Canterbury Health Laboratories**

***K J Moore, A Beddowes. Canterbury Health Laboratories, Christchurch and Central Regional Genetic Services, Wellington Hospital, Wellington***

Open system coverslip culture and in situ harvesting of amniotic fluid cells is a well established protocol. The advantages of this technique are the straightforward differentiation between true and pseudomosaicism and the rapid processing times due to enhanced cell proliferation. Twenty amniotic fluid specimens were processed in tandem with the Cytogenetics laboratory at Central Regional Genetic Services, Wellington Hospital. Subsequent analysis was performed in accordance with NPAAC (2001) guidelines; all samples processed fulfilled the necessary criteria with a minimum QA score of 4 (range 4-5) and a mean reporting time of 10.3 days (range 7-15 days). Following the success of this study, Canterbury Health Laboratories has now incorporated this technique into their diagnostic cytogenetics service.l

**Editor's note:** As per editorial policy, any abstract submitted to the ASM containing the phrases: "results will be presented" or "results will be discussed" have been omitted or have had these phrases deleted.

**Answers to HSIG Journal questionnaire:**

1. The need to transport oxygen and remove carbon dioxide from the tissues.
2. 10 years after the menopause.
3. 90%
4. 14.8mg.
5. In the mitochondria.
6. Dairy produce, cereals and tannin.
7. Catecholamiones.
8. Lower IQs, decreased neutrophil function in response to infection, reduced work capacity and performance, reduced thermoregulation, hair loss.
9. The early 20s
10. The current normal values for red cell count haemoglobin and ferritin were obtained from sampling populations that contained a large proportion of women with iron deficiency.
11. Using the reference ranges for men for these parameters when assessing the haematological and iron status of women in order to bring positive implications for women's health and welfare.

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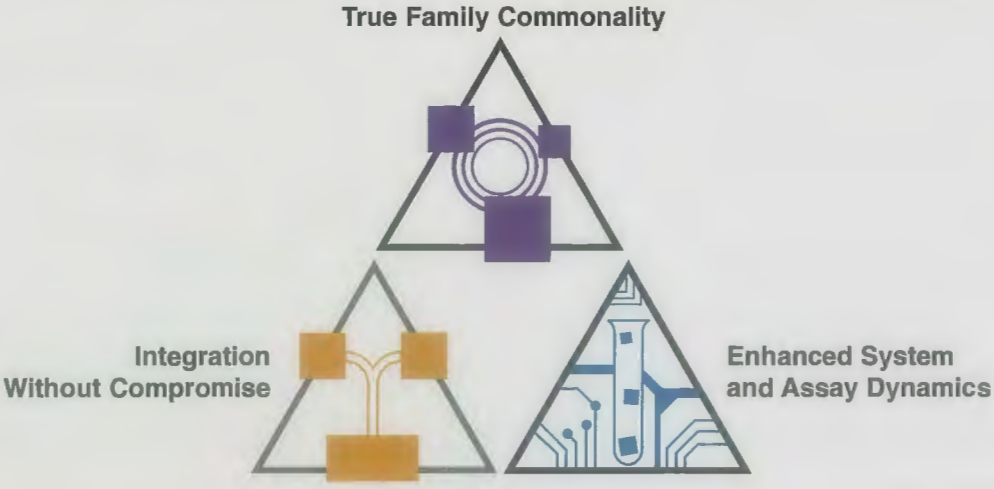


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