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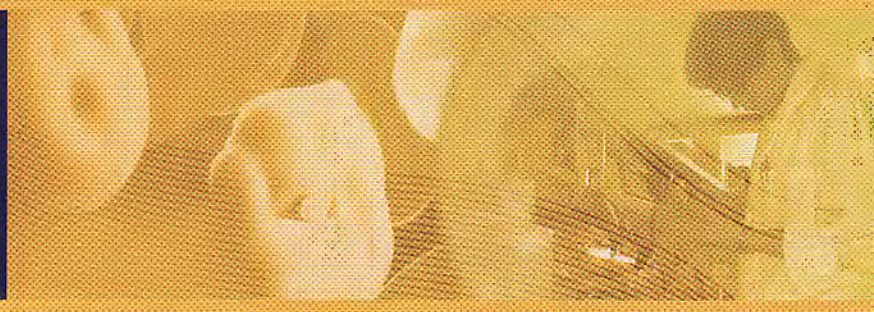

ISSN 1171-0195 Volume 60 Number 2 August 2006

60 years in publication

New Zealand Journal of Medical Laboratory Science

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

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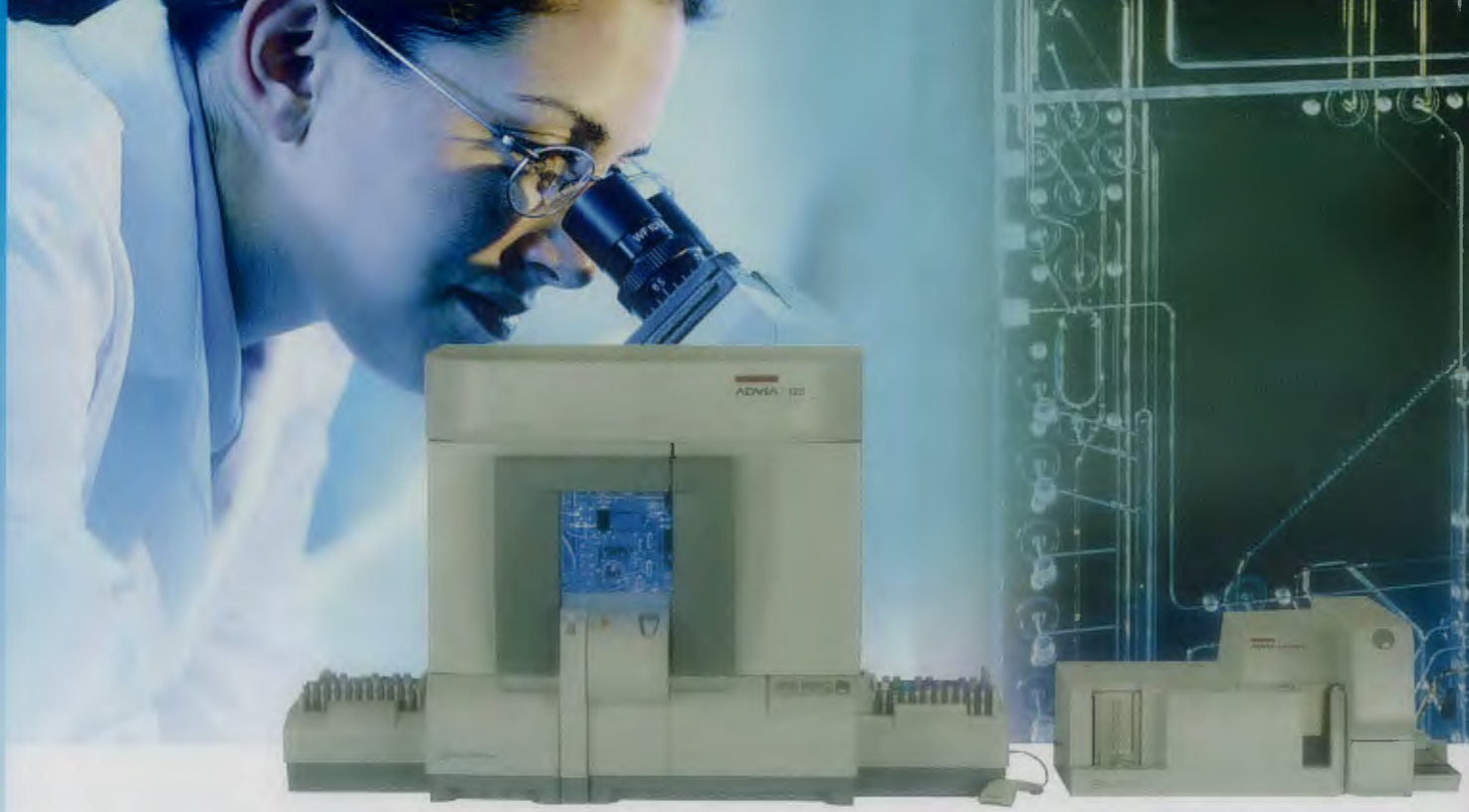

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 The Journal is published three times per year in April, August and November, printing by Centurion Print, PO Box 6344, Wellesley St., Auckland.

The Journal is abstracted by the Cumulative Index to Nursing and Allied Health Literature (CINAHL), Index Copernicus, Excerpta Medica/EMBASE, and the Australian Medical Index. The journal, through its Editor, is a member of the World Association of Medical Editors (WAME).

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New Zealand Journal of
Medical Laboratory Science
Volume 60 Number 2
August 2006
ISSN 1171-0195

Editorial

The NZIMLS & the MLSB - who does what?

If you are one of the many within the profession who are confused about the roles of the New Zealand Institute of Medical Laboratory Science (NZIMLS) and the Medical Laboratory Science Board (MLSB) then the following article may help. It presents a general overview of these two organisations and covers the roles of these two organisations in the administration of the profession in New Zealand.

NZIMLS

Mission statement

"The New Zealand Institute of Medical Laboratory Science is the professional organisation that represents those engaged in the profession and practise of Medical Laboratory Science in New Zealand. It has an ongoing commitment to promote professional excellence through communication, education and a code of ethics to achieve the best laboratory service for the benefit of the patient".

The NZIMLS is therefore the body that serves the professional needs of both Medical Laboratory Scientists and Technicians in New Zealand. The NZIMLS is administered by an elected Council, which is comprised of five regional representatives, a Vice-President, Secretary/Treasurer and the President. Those elected to Council have assigned portfolio responsibilities such as professional affairs, finance, promotion, CPD, education, SIG's etc. Under the rules of the organisation all officers stand down each year at which time nominations for positions on Council are sought from the Membership. Any financial Member of the organisation can be nominated for the seats on Council. The results of the elections are announced each year at the Annual General Meeting, which is usually held in conjunction with the Annual Scientific Meeting (ASM). Each of the Council positions is honorary, with those elected giving up their own time to administer the affairs of the profession.

In addition to the elected Members of Council, the NZIMLS contracts with others to provide other important services offered by the Institute. These contracts include the positions of Executive Officer (Fran van Til), CPD Programme Coordinator (Jillian Broadbent) and Advertising Manager (Trish Reilly). Council meets with the Executive Officer, CPD programme Coordinator and the Editor four times a year. At these meetings Council discusses matters raised by its Members, finances, planning, examinations, Journal, CPD and approves applications for Membership. Running costs of the Institute are met from income derived from membership levies, CPD programme membership, and scientific meetings held under the aegis of the NZIMLS. Membership of the NZIMLS is voluntary and currently there are approximately 1900 members in all categories of membership.

Function

The NZIMLS concerns itself with the professional affairs of its members and plays no role in negotiations with employers over the terms and conditions of employment of its Members. The NZIMLS has as its major focus, the provision of continuing education and the provision of a competency and professional development programme to meet the needs of its Members. Ongoing education in the profession is provided by the Institute through the ASM, and the many special interest group (SIG) meetings held throughout NZ each year. The Institute also offers professional examinations (Fellowship, QMLT, QPT and QSST), publishes a journal three times a year, and acts as caretaker of the educational standards of the profession in NZ. This is achieved through Institute positions on university Board's of Study or Management Committees. The NZIMLS has ongoing dealings with other health-related organisations in NZ, government departments, the MLS industry and kindred overseas organisations. As part of its role the NZIMLS is often consulted by the MLSB over proposed regulations governing the profession or other MLS matters that may impact upon the public of NZ.

Confused roles

The following are a few examples of the ongoing confusion that exist among over the services offered or jurisdictions of the NZIMLS and the MLSB:

- The SIG's have been setup by the NZIMLS and their ongoing activities receive financial and personnel support from the NZIMLS,
- SIG meetings are NZIMLS activities,
- The NZIMLS does not approve applications for registration in NZ,
- The NZIMLS does not issue the Annual Practising Certificate,
- The NZIMLS provides the CPD programme, not the MLSB,
- It is the MLSB who requires you to participate in a re-certification programme such as the CPD programme.

NZIMLS Office

The headquarters of the NZIMLS is located in Rangiora and the Executive Officer of the NZIMLS should be the first person for you to contact for any enquiries about the activities described in this editorial. Fran will be happy to assist you with any queries about Membership, examinations, or scientific meetings or be able to direct you to the person who may be able to help. For further information on the NZIMLS visit www.nzimls.org.nz or contact:

Fran van Til
PO Box 505
RANGIORA
Tel: (03) 313 4761
Email: fran@nzimls.org.nz

MLSB

Composition

The Medical Laboratory Science Board (MLSB or the Board) is a statutory body appointed by the Minister of Health to regulate the profession of medical laboratory science under the Health Practitioners Competence Assurance Act. This Act ensures that every registered health practitioner in New Zealand is operating under the same legal rules and provisions.

The Board is currently made up of eight members: five registered scientists, one pathologist and two lay members of the public. We have asked the Minister of Health for the Board numbers to be increased to include a Medical Laboratory Technician. The appointments are for

a three-year term and while members can be reappointed they can only serve for a maximum of three terms. A full time Registrar (Phil Saxby) and Deputy Registrar (Mona Forrester) support the Board in its activities.

Function

The fundamental purpose of the HPCA Act is conveyed in the following statement: "The principle purpose of the Act is to protect the health and safety of members of the public by providing for mechanisms to ensure that health practitioners are competent and fit to practice their professions", (see page 7 of the Act).

To achieve this aim, the Board must carry out several primary functions:

- Prescribe the qualifications required for scopes of practice within the profession,
- Accredit and monitor educational institutions and qualifications,
- Authorise the registration of medical laboratory scientists and medical laboratory technicians and to maintain registers,
- Consider applications for annual practicing certificates,
- Review and promote the competence of medical laboratory scientists and medical laboratory technicians,
- Recognise, accredit and set programmes to ensure the ongoing competence of medical laboratory scientists and medical laboratory technicians,
- Receive and act on information about the competence of medical laboratory scientists and medical laboratory technicians,
- Consider the cases of medical laboratory scientists or medical laboratory technicians who may be unable to perform the functions required for the practice of the profession,
- Set standards of clinical competence, cultural competence and ethical conduct to be observed by medical laboratory scientists or medical laboratory technicians.

Most of the MLSB functions are clearly delineated from those of the New Zealand Institute of Medical Laboratory Science but there are two areas where there is considerable interaction.

1. Consultation

When the Board sets policies and guidelines on significant issues it consults with the profession. As the majority of the profession belong to the New Zealand Institute of Medical Laboratory Science (NZIMLS) the Institute Council (your elected representative group) is an important contributor to the MLSB's consultation processes.

2. Monitoring competence for re-certification

The greatest area of confusion over the roles of the MLSB and the NZIMLS is related to the monitoring of competence. As mentioned above, one of the primary functions of the Board is to monitor the competence of registered scientists in order to ensure the protection of the public. A well-designed and administered re-certification program provides a mechanism for the Board to achieve this requirement and when the HPCA Act came into force, the Board gave its approval to the Continuing Professional Development (CPD) programme of the NZIMLS. No other programmes have been approved to date. A condition of its approval is that the programme must be offered to all registered medical laboratory scientists in New Zealand.

The NZIMLS administers the CPD programme and provides audit reports to the Board. All questions about the operation of the CPD programme should first be directed to the NZIMLS.

For scientists, the MLSB requires evidence of satisfactory progress in the CPD programme (unless exempted) in order to be sure that competence is being maintained. When applying for an Annual Practicing Certificate, practitioners need to attach a copy of their CPD history to the application form. The Board does not have access to the CPD histories except by special request, and does not receive copies submitted to the audit process.

So to help clarify the relationship here is a summary of situation

- The MLSB requires all scientists to belong to an approved recertification programme.
- The NZIMLS has an approved programme (CPD), which it administers for the profession.
- Scientists log their points into the NZIMLS CPD program.
- Scientists must provide a CPD history to the MLSB when applying for an APC.

MLSB Office

Recently the MLSB and the Medical Radiation Technologists Board formed a joint company called Medical Sciences Secretariat to provide administrative services to both boards. The company consists of four registrars (two for each board), a manager and an office administrator and is located in Wellington.

Phil Saxby, the Registrar, can be contacted through the office or by email: phil.saxby@medsci.co.nz or mls@medsci.co.nz

Contact details for the office are:
PO Box 7242
Wellington South
Tel: 04 801 6250
www.mlsboard.org.nz

Chris Kendrick, President of the NZIMLS
Ross Anderson, Chairman of the Medical Laboratory Science Board

In this issue

In the Editorial by the President of the New Zealand Institute of Medical Laboratory Science (NZIMLS) and the Chairman of the Medical Laboratory Science Board (MLSB), Chris Kendrick and Ross Anderson explain the roles of the respective organisations and more importantly the differences between the roles of the NZIMLS and the MLSB.

In the article by the Wellington Asthma Research Group, the authors review their published findings on house dust mite allergens and allergic diseases. They have found, among other, that New Zealand has some of the highest house dust mite allergens in the world, that synthetic pillows contain substantially more house dust mite allergens than feather pillows, and that Tokelau is a virtual house dust mite allergen free environment.

Lesley Newton from the Canterbury Health Laboratories presents an unusual case of septicaemia caused by *Capnocytophaga canimorsus*. This Gram-negative anaerobic fusiform bacillus is normally present in saliva from cats and dogs. In this case the organism was present intracellularly on examination of the blood film.

In this issue is reprinted a historical article by Hugh Bloore, formerly principle technologist at Wairau Hospital and previous President of the Institute. Hugh passed away last year and his family have kindly donated funds for the Hugh Bloore poster prize, the first to be awarded at the Napier ASM in August. In his article from 1960, Hugh explains the principles and methods for blood gas measurement using the Astrup apparatus, the first instrument of its kind in the world. In those days a complete blood gas analysis required 12-13 ml of blood and took 12-13 minutes to analyse, a far cry from today.

As well as the historical article by Hugh Bloore, this issue also reprints the minutes of the Institute's council meeting of 1946, and a historical advert from the leading medical bookseller in New Zealand, NM Peryer Ltd., now known as Medical Books Ltd. This advert was for the latest edition of the classical textbook entitled Chemical Methods in Clinical Medicine by Harrison. At the council meeting of 1946, council members discussed at great lengths the proposed introduction of a new syllabus of training for hospital bacteriologists, as we were known then.

Also in this issue a new journal-based questionnaire based on the entire contents of the Journal. Getting at least 7 out of 10 questions right earns you 5 valuable CPD points. By taking part in all 3 issues published per year can earn you 15 of the other 40 CPD points needed for the year (37.5%) without leaving the lab or home. Please note that submission of answers is via the NZIMLS web site, no fax or mail please.

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.

The winner of the Med-Bio Journal Prize from the April 2006 issue was Christiaan Sies from Canterbury Health Laboratories, Christchurch for the article "A guide to the diagnosis of porphyria: suggested methods and case examples". *N Z J Med Lab Sci* 2006; 60 (1): 7-11.

House dust mite allergens and allergic diseases - the Wellington Asthma Research Group studies

Rob Siebers, PGCertPH, FNZIC, FNZIMLS, Senior Research Fellow;
Kristin Wickens, BA, DPH, PhD, Senior Lecturer;
Julian Crane, FRACP, FRCP, Professor of Clinical Epidemiology

Wellington Asthma Research Group, Department of Medicine, Wellington School of Medicine and Health Sciences, Wellington

Abstract

Allergens produced by house dust mites are known to induce sensitisation in susceptible subjects and in turn sensitisation is associated with the development of allergic asthma. Furthermore, exposure to house dust mite allergens is an established risk factor for exacerbation of allergic asthma.

In this paper we review published studies from the Wellington Asthma Research Group on house dust mite allergens over the last decade. These studies have shown that New Zealand has some of the highest levels of house dust mite allergens in the world with extremely high levels of allergens present in the new born infant's environment; Tokelau is a virtual house dust mite allergen free environment; synthetic beddings contains significantly higher allergen levels than feather beddings due to their permeability to house dust mites; carpets are a significant reservoir for allergens; and domestic clothes dryers can significantly reduce house dust mites from duvets.

These studies from the Wellington Asthma Research Group have contributed significantly to the international literature on house dust mite allergens and have included novel findings.

Key words: house dust mites, asthma, allergy, allergens, bedding, pillows, risk factors, New Zealand.
N Z J Med Lab Sci 2006; 60(2): 49-58.

Introduction

For centuries house dust has been known to lead to allergy and asthma symptoms in susceptible individuals. The Flemish physician, John Babtista van Helmont in 1662 described such symptoms in one of his patients *"...as oft as any place is swept or the wind doth otherwise stir up the dust, he presently falls down, being almost choked"*. In the 1960s researchers in the Netherlands and Japan independently showed that house dust mites belonging to the *Pyroglyphidae* family, namely *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae,* were major sources of allergen in house dust (1,2).

Like spiders, house dust mites belong to the class *Arachnida*. Of at least 50 species of house dust mites that have been found in domestic house dust, the two mites of the family *Pyroglyphidae*, namely *D. pteronyssinus* and *D. farinae*, are the most important in temperate climates, both in terms of numbers and of clinical relevance. Clinically, these house dust mites are of relevance because most asthma symptoms in children and young adults are associated with both an immediate hypersensitivity to their inhaled allergens, and a familial tendency towards atopy (3).

Asthma is a major clinical problem worldwide and there is evidence that both prevalence and severity of asthma are increasing (4,5), although some recent evidence points to a halting or decline in asthma prevalence in some countries (6). In New Zealand, 30% of 13-year-old children are sensitised to house dust mites, and that sensitivity is a significant independent risk factor associated with the development of asthma (7). Thus it is not surprising that increased focus has been directed towards the link between house dust mite allergens and asthma and allergy over the last couple of decades. Indeed, a Medline search from 1985 to 2005 returned 2,139 articles on house dust mites, an average of about two articles per week.

House dust mites are about 1/4 to 1/3 mm long, are eight legged, have no eyes, and have no developed respiratory structures. External temperature controls their body temperature, and their moisture requirements are regulated by passive and active mechanisms, mainly through saturated KCl and NaCl channels that extract water from moist air and prevent body moisture loss during low humidity conditions (8). Figure 1 shows an adult *D. pteronyssinus* which is the dominant mite species found in New Zealand homes (9), although we have found evidence of allergens from *D. farinae* in New Zealand, but at much lower levels than those from *D. pteronyssinus* (10).

The house dust mite *D. pteronyssinus* produces at least seven major allergens (and another 7 minor allergens) officially recognised by the WHO/IUIS Allergen Nomenclature Subcommittee (11). The major group one allergen, Der p 1, has been the most studied allergen of this house dust mite over the years. It is a 25 Kda protein showing homology with cysteine proteases (12). Very large quantities of Der p 1 are found in house dust mite faeces, its nature reflecting the digestive processes of house dust mites through the enclosure of food and cell debris by a peritrophic membrane (13). House dust mite allergens are widely distributed in the domestic environment. They are found abundantly in carpets, furniture, mattresses, pillows, duvets, blankets, curtains, soft toys, clothing, and are even detectable in human hair (14), on human skin (15), and in cord blood (16).

Over the years, studies have consistently shown that sensitisation to house dust mite allergens is strongly associated with asthma in children and adults (17,18), that the development of atopy and asthma follows early childhood exposure (19), strict avoidance to house dust mite allergens reverses asthma symptoms (20,21), and that there is a dose-response relation between Der p 1 exposure and the severity of asthma in house dust mite sensitised children (22).

To prevent predisposed infants from developing atopy and subsequently asthma, and to reduce symptoms in house dust mite sensitised asthmatics, various techniques have been trialled to kill house dust mites and to reduce their allergens in domestic sites, with mixed results. These techniques have included reduction of indoor humidity by mechanical ventilation and heat recovery (23,24), chemical treatment of carpets, bedding and furniture with acaricides such as benzyl benzoate and tannic acid to denature allergens (25,26), intensive vacuum cleaning (27), use of barrier covers on bedding (28,29), exposure to direct sunlight (30), super-heated steam cleaning (31), liquid nitrogen (32), washing and dry cleaning of bedding (33), and hot tumble drying of duvets (34).

In this article we review studies undertaken and published by the Wellington Asthma Research Group on house dust mite allergens over the last decade. These studies have contributed significantly to the international literature and have included novel new findings.

Methodological aspects

Early methods for quantification of Der p 1 (originally termed antigen P_1) included radio allergen sorbent inhibition, counter immuno-electrophoresis and radioimmunoassay (35). These techniques were useful in assaying mite allergen levels in areas where *D. pteronyssinus* is the dominant mite species, but not where *D. farinae* is dominant, due to partial cross-reactivity between Der p 1 and Der f 1 (the major group one allergen of *D. farinae*). In order to overcome cross-reactivity with the major group two allergens of the *Dermatophagoides* spp., double-monoclonal antibody radioimmunoassays specific for Der p 1 and Der f 1 were developed and subsequently adapted to ELISA (36).

House dust mite allergen detection and quantification by ELISA has the advantage of being a non-isotopic immunoassay readily adaptable by most laboratories for use in epidemiological and interventional studies of house dust mite allergens and is the method of choice by the majority of research laboratories active in this field. An American-based company, Indoor Biotechnologies (Charlottesville, Virginia, USA) is the major manufacturer of these double-monoclonal antibody ELISA kits for the detection of Der p 1 and Der f 1. Additionally, the company also produces ELISA kits for the detection of *Dermatophagoides* group two allergens (from both *D. pteronyssinus* and *D. farinae*) and for the detection of the major allergen from the tropical house dust mite, *Blomia tropicalis*, which is the dominant mite species in tropical and semi-tropical areas (37). The Second International Workshop on House Dust Mite Allergens and Asthma set threshold levels for Der p 1 of 2 μg/g dust and 10 μg/g dust respectively for sensitisation in predisposed infants and acute exacerbation of asthma (38) although sensitisation can develop at levels as low as 0.5 μg/g dust (39).

House dust samples are extracted and the allergen quantified by double-monoclonal ELISA. In the 1990s, at the request of the Third International Workshop on Indoor Allergens and Asthma (12) we set up an external quality control program for Der p 1. After two rounds, in which two dust samples were sent to 23 laboratories worldwide, a six to seven-fold variability in Der p 1 results was apparent (40). In round three we sent six dust samples with a wide range of Der p 1 concentrations to these laboratories to test whether mean results from multiple samples were similar, or not. Results from this round re-confirmed the wide variability in results (41). This would imply that comparing Der p 1 levels between laboratories might not be valid. It also became apparent that laboratories extracting in the cold returned lower Der p 1 results than those extracting at room temperature. There also seemed to be differences in results depending on the buffer being used for extraction. This led us to study the effects of temperature and buffers on the extraction of Der p 1 from house dust (42). That study showed a mean reduction in Der p 1 levels from dust extracted at 4°C of 57.9% compared to room temperature extracts, and dust extracted with borate-buffered saline had Der p 1 values approximately twice the levels from phosphate-buffered saline or ammonium bicarbonate buffer, independent of pH (between 7.0 and 8.5) or ionic strength. Thus, when comparing results from different research centres, differences in extraction techniques must be taken into consideration.

Dust for house dust mite allergen analysis is usually collected by vacuuming. In order to present results as both an amount of allergen per amount of dust and a concentration per unit area (recommended for exposure assessment), standardised collection techniques are required. We usually collect dust samples by vacuuming a 1 m^2 area for 1 minute using the same brand vacuum cleaner for all studies. As uncarpeted floors generally results in small quantities of dust, we sample a 2 m^2 area from smooth floors. For irregular surfaces, such as furniture or pillows, the whole item is vacuumed for a set time, but for these items Der p 1 results can only be expressed as absolute concentrations but not as a concentration per unit area.

A previous study demonstrated a wide variation of Der p 1 levels within living room and bedroom floors (43). This has implications for epidemiological studies as normally a central area within the room is sampled with the assumption that this is representative of the whole room. As that study was conducted in the UK, where Der p 1 levels are generally 20-fold lower than in New Zealand, we determined the variability of Der p 1 (and of the cat allergen, Fel d 1) in domestic living room floors in Wellington (44). Mean coefficient of variation for Der p 1 from living room floors was 53.1% (range: 28.5-136.8), therefore a single sample from a floor is not suitable to assess an individual's exposure risk but is representative of the whole room in large-scale epidemiological studies.

Vacuumed dust samples frequently contain larger particles of numerous origins; therefore dust samples are normally sieved before analysis. We sieve through a 425 μm steel mesh sieve to obtain fine dust. When collecting dust from pillows, normally only small quantities of fine dust are obtained. We therefor determined the effects of sieving on Der p 1 levels (45). Dust was collected from 24 living room floors and 24 mattresses, the dust samples split into two lots with one lot being sieved. Bland-Altman plots showed that, although yielding slightly lower Der p 1 levels, not sifting dust did not result in empirically different Der p 1 levels. Sieving dust takes time and may be an additional factor contributing to the total inaccuracy inherent in Der p 1 measurement.

As well as differences in sampling times and areas sampled, and use of vacuum cleaners with different wattage, there are also various dust collection devices in use that may influence dust yield and thus Der p 1 levels. We use a nylon mesh bag (25μm pore, developed by Dr. E. Tovey, Sydney) inserted between the vacuum hose and the vacuum cleaner furniture attachment to collect dust. We are also involved in the International Study of Allergies and Asthma in Childhood (ISAAC) where for phase 2 centres around the world are collecting dust in a standardised manner using dust collection devices (ALK, Copenhagen, Denmark) consisting of a filter dish pre-loaded with 70 mm Whatman no.4 25μm filter paper. We collected duplicate dust samples from 37 carpeted living room floors and from longitudinal halves of 37 mattresses with the two dust collection devices using the same vacuum cleaner. These dust samples were analysed for Der p 1 (as well as for

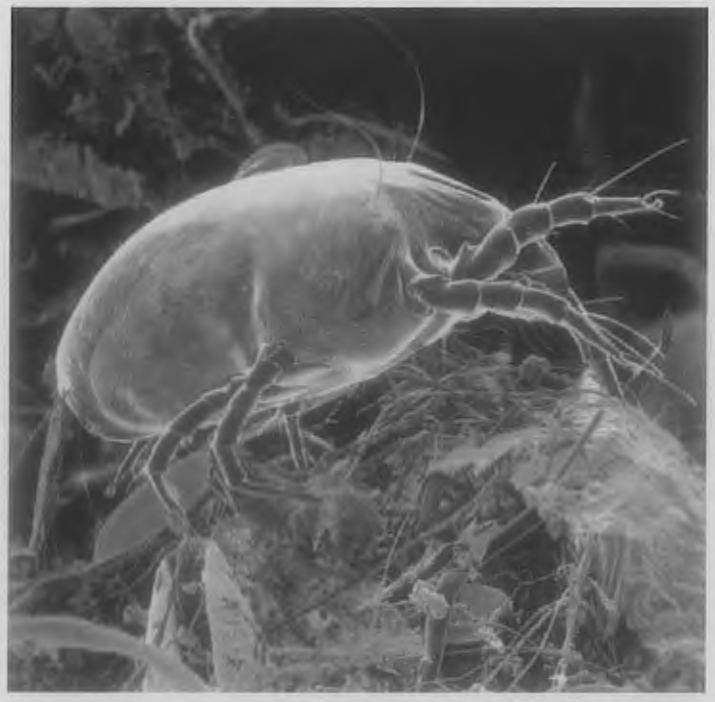

Figure 1. The house dust mite *Dermatophagoides pteronyssinus*

cat allergen and bacterial endotoxin). Results showed that the use of nylon mash bags resulted in more dust and thus higher absolute concentrations of Der p 1 than with the ALK device (46). Floor Der p 1 levels, expressed as μg per gram of dust, were also significantly higher using nylon mesh bags, but this was not so for mattress samples. Thus, in order to have confidence that the comparison of Der p 1 levels between centres is valid, not only Der p 1 analysis methodology, but also the standardisation of dust sampling equipment is essential.

Although double-monoclonal antibody ELISA remains the gold standard for measuring Der p 1, it is essentially a research tool and relatively expensive due to labour intensive collection and preparation of the dust samples. Various simple 'dipstick' semi quantitative systems have been developed to allow homeowners to test their house dust for house dust mite and other allergens. One such system has recently been developed by Indoor Biotechnologies, the major manufacturer of the ELISA Der p 1 research kit. It is a simple rapid test (30 to 60 min) using lateral flow technology and gold-labelled monoclonal antibodies to Der p 2, and can thus detect allergens from both *D. pteronyssinus* and *D. farinae*. We took part in a multi-centre evaluation of this rapid test. Archived dust samples (n=349) from homes in nine centres from eight countries (the Netherlands, USA, Brazil, Sweden, France, UK, Australia, New Zealand) were analysed for group 1 and group 2 house dust mite allergens and also compared with the rapid test (47). Significant correlations were obtained between Der p 1 and Der f 1 with Der p 2, with the strongest correlation from New Zealand. Significant differences were obtained between Der p 2 levels and the three rapid test scores (negative to low; low to medium; medium to high). The rapid test also showed a low rate (3.15%) of false negative reactions. The rapid tests also contain a convenient sampling and extraction device, allowing allergic patients to simply and rapidly test for house dust mite allergen exposure in their homes. The company has now developed 'credit card' systems using the lateral flow technology and gold-labelled antibodies for simultaneously detecting allergens from house dust mites, cat, dog and cockroach.

House dust mite allergens and the indoor environment

As previously mentioned, house dust mite allergens in the domestic environment can come from many sources. Over the years we have conducted a number of studies of house dust mite allergens in the indoor environment in New Zealand. Our first major study involved measuring Der p 1 on living room and bedroom floors and on mattresses of 474 children in Wellington (48). This study showed that Wellington has some of the highest house dust mite allergen levels in the world, and that higher floor Der p 1 levels were associated with older carpets and the presence of more than two children. Bedding Der p 1 levels were higher in beds with kapok and inner sprung mattresses, those with woollen under lays, and high relative humidity on the mattress. Reducing exposure to the very high levels of house dust mite allergen in New Zealand will be a major challenge, perhaps the most important being the removal of carpets and occlusive covering of bedding.

Having determined that carpets, especially older carpets, were the most and important determinant of floor Der p 1 levels, we set out to determine which housing characteristics explain Der p 1 variability. We re-sampled a subset of these houses with carpeted living room floors and selected those with the highest and lowest Der p 1 levels (49). The main findings were that lower levels of Der p 1 were associated with floor insulation, a thick layer of underlay, and the presence of more than two children. Also, although previously higher, Der p 1 levels had not significantly changed over a four-year period.

Having determined the levels of house dust mite allergens in the indoor domestic environment in Wellington, we were interested to see what the levels were in other indoor environments in New Zealand. In collaboration with the Canterbury Respiratory Research Group (Christchurch) we collected floor, bed and seat dust samples from hotels, hospitals, rest homes primary schools, child care centres,

cinemas, churches, ski lodges, banks, and airplanes (50). We found that Der p 1 levels were significantly lower in these public places, compared to the domestic environment. Although much lower, we again confirmed that, even in public places, carpets were a significant source of house dust mite allergens, and lower Der p 1 levels were associated with frequency of cleaning.

House dust mite allergens and the bed room

We spend approximately one third of our lives in bed. Given its close proximity to the airways, bedding and mattresses are important sources of house dust mite allergen exposure. As exposure to Der p 1 is an important determinant of allergic sensitisation in the first year of life, we measured Der p 1 levels in infant bedding in Wellington. Bedding dust samples were collected from 154 newborn infants at a mean age of 11 weeks and again at a mean age of 15 months (51). At 11 weeks bedding Der p 1 levels were high, being approximately 10-fold higher than levels reported in other countries, and these levels increased significantly at 15 months. Bedding that included a sheepskin (used by a third of the infants) was associated with the highest levels of Der p 1. Thus in New Zealand newborn infants are exposed to levels of Der p 1 much higher than has been associated with sensitisation and exacerbation, and given these high levels it is not surprising that asthma in New Zealand is common, severe and dominated by house dust mite allergy.

It has been known for some time that sheepskins harbour house dust mites (52,53). Given the high levels of Der p 1 on sheepskins, we were interested in seeing how quickly new sheepskins accumulate house dust mite allergens, and the effectiveness of both washing and dry cleaning on its removal (54). Newly bought sheepskins were placed in the domestic environment (floors and mattresses) and monitored for Der p 1 accumulation over six weeks. They were then warm-washed, sampled for dust, returned to the same floors and mattresses for a further six weeks and then dry-cleaned and re-sampled. Der p 1 levels rose rapidly over time, while warm washing and dry-cleaning reduced sheepskin Der p 1 levels by 79.2% and 95.3% respectively. Thus, sheepskins rapidly accumulate house dust mite allergens and should be discouraged as infant bedding for those at risk of developing sensitisation. If used, they should at least be regularly washed or dry-cleaned.

House dust mite allergens, and synthetic and feather bedding

For many years allergic patients with asthma, rhinitis and eczema have been advised to avoid feather pillows on the assumption that these are a source of large amounts of house dust mites as a great number of mites are found on bird feathers (55). Indeed, *pteronyssinus* means feather loving. However, modern pillow manufacturing processes ensure that through superheat steaming of feathers used for pillows and duvets, no mites survive and their allergens are denatured (56). In 1995 a study showed that the use of synthetic pillows was a significant indoor environmental factor associated with severe asthma in children, while feather pillows appeared to be protective (57). The authors hypothesised that synthetic pillows may release organic volatile compounds that would adversely affect the airways of children. We hypothesised that their findings could be due to differences in house dust mite allergen levels between different types of pillows. We set out to test this by measuring Der p 1 levels on pillows and collected dust from pairs of synthetic and feather pillows placed on the same bed for more than six months (58). To our surprise we found seven to eight-fold higher levels of Der p 1 on synthetic, compared to feather pillows. Our findings, though interesting, were met with some scepticism in the research community. However, our English colleagues, at our urging, found the same applied in the UK, where house dust mite allergen levels are about 20-fold lower than in New Zealand (59). They also found that feather pillows contained much lower levels of cat and dog allergens than synthetic pillows.

We then set out to determine at what rate new synthetic and feather pillows accumulate house dust mite allergens. We placed 12 pairs of pillows, each pair consisting of a feather and synthetic pillow, on 12 mattresses and collected dust regularly over a 12-month period (60). After 12 months synthetic pillows contained on average five times as much Der p 1 than feather pillows, confirming our previous results (58) and those from the UK (59). The accumulation rate of Der p 1 on both types of pillows is governed by the environment, as there was a significant correlation between pillow Der p 1 accumulation and Der p 1 levels of the mattresses they were placed on. These findings attracted several leading Editorials/Reviews, including from our research group (61-63).

We hypothesised that the differences in allergen levels between pillows could be related to the weave of the pillow covers as we had previously noted that the weave of the covers on feather pillows was much tighter than those on synthetic pillows (64). When contacted, a leading New Zealand manufacturer said the reason for the tighter weave of feather pillow covers was to keep the feathers inside the pillows. We therefore undertook a study to determine the permeability of feather and synthetic pillow covers to house dust mites and house dust (65). We seeded 20 live adult house dust mites on top of feather and synthetic pillow covers with adequate food supply underneath in sealed culture dishes kept at room temperature and high humidity. After 24 h all mites had penetrated the synthetic pillow covers while after 48 h no mites had penetrated the feather pillow covers. Dust permeability of the synthetic pillow covers was more than 12 times greater than that of the feather pillow covers. We believe these results provide a convincing explanation for differences in house dust mite allergens between feather and synthetic pillows.

The use of synthetic duvets has increased significantly over recent years. As the majority of these in use in New Zealand are synthetic, we undertook a study to look at house dust mite allergen levels in individual bedding items. We collected dust samples from 65 duvets, 81 pillows and 65 mattresses from 34 children and 31 adults in Wellington (66). As well as again showing that synthetic pillows contained much higher Der p 1 levels than feather pillows, synthetic duvets also contained much higher Der p 1 levels, 15-fold higher than feather duvets. This study, published in the *New Zealand Medical Journal* attracted intense media attention with newspaper articles throughout the country, radio interviews, and the lead story on TV 1.

Two further pillow studies were undertaken. The first one was in conjunction with Korean colleagues. In Korea, buckwheat pillows are commonly used. We determined house dust mite allergen accumulation on new buckwheat and synthetic pillows in Korea. As the dominant house dust mite species in Korea is *D. farinae* we measured the major group one allergen, Der f 1, from this mite species. We found no difference in Der f 1 accumulation between the two different pillow types but the amount of endotoxin (from Gram-negative bacteria) from buckwheat pillows was approximately 12-fold higher compared to synthetic pillows (67). Thus buckwheat pillows may affect asthma severity as endotoxin is a pro-inflammatory compound that exacerbates asthma in house dust mite sensitised asthmatics (68).

In another study we determined the effects of lavender on house dust mite allergens in pillows as lavender oil is known to be acaricidal (69). Six new pairs of pillows, each pair consisting of identical synthetic pillows with and without a lavender sachet inside were placed on mattresses and Der p 1 accumulation studied over three months. Der p 1 accumulation was similar between the two types of pillows; thus, addition of lavender sachets (which smells nice and is supposed to have a soothing effect) inside pillows is unlikely to be a beneficial house dust mite reduction measure.

House dust mite allergens in other environments

House dust mite allergens are distributed throughout the home with the main exposure from floors and bedding. However, there are potentially other exposure routes. Tovey et al previously reported that adult clothing was an unrecognised source of allergen exposure (71). We studied house dust mite allergen levels of upper garments worn by 166 school children in Wellington (72). Although levels of Der p 1 were lower in clothing than normally found in the indoor environment, some still had levels associated with the exacerbation of asthma. Interestingly, we found that girls clothing had higher levels of Der p 1 than boys clothing. Perhaps boys clothing is washed more frequently or girls spend more time in direct contact with house dust mite allergen laden carpets and bedding. Also, the type of garment influenced Der p 1 levels, with woollen garments containing the highest levels. Thus house dust mite sensitised asthmatics should perhaps avoid woollen garments.

A report from Brazil suggested that human heads were an unrecognised reservoir of house dust mites (73). Their published data reported the number of mites per gram of vacuumed scalp dust. As the amounts of dust collected from scalps was small, we calculated that there was on average two mites per scalp, unlikely to be a major source of house dust mite allergens. We tested this out by collecting vacuumed dust from hair of 16 adults from our research group (14). House dust mite allergens were present in these samples, but at very low levels and thus human hair is unlikely to be a significant source of house dust mite allergen exposure.

Subjects with atopic dermatitis are frequently sensitised to house dust mites and reduction of exposure to its allergens in bedding has been shown to be of benefit (74). We wondered whether there were significant amounts of house dust mite allergen on the skin surface after rising from bed. We asked 25 subjects to vacuum their entire body after getting up and shedding any bedclothes, and also collected dust from their bedding (15). We found that, although the levels of Der p 1 on skin were low, these levels are known to elicit skin responses in atopic dermatitis. The major determinant of skin Der p 1 was bedding Der p 1.

House dust mites need a temperate climate and relative humidity levels of above 45% to proliferate and survive. This is why house dust mite allergen levels are very high in the temperate climates of New Zealand and Australia, compared to colder winter climates such as in the Netherlands (48, 75-76). We were interested to see if house dust mite allergens could be detected in the Antarctic, where outdoor relative humidity rarely rises above 20% and thus is not conducive for house dust mite survival. We hypothesised that any detectable allergen levels there would most likely to have been introduced passively from clothing. We obtained dust samples from the clothing of 11 recently arrived Scott Base personnel as well as from their mattresses and three living room areas (77). House dust mite allergens were undetectable in the living room areas and in all but one mattress (at a very low level). Six of the 11 sweaters from the Scott Base personal had detectable Der p 1 levels, albeit at low concentrations. This study confirmed passive transfer of house dust mite allergens in an area devoid of sustainable house dust mite populations. Interestingly, we also found significant levels of passively transferred cat allergen in the Antarctic, an area totally devoid of cats.

Many university students live in low cost, poorly maintained rental accommodation or in halls of residence with no choice of the type of bedding or floor coverings. We were interested to see whether university students were exposed to higher levels of house dust mite allergens. Dust samples were collected from bedroom floors and mattresses of 178 1st year students at the University of Otago in Dunedin (78). Student accommodation was grouped into family homes (n=61), student flats (n=43) and halls of residence (n=74). The highest levels of Der p 1 were from family homes, followed by student flats and halls of residence. The lowest levels found in halls of residence possibly is due to regular cleaning and washing of bedding in this type of student accommodation, and the vacation of these premises for three month over summer. Domestic Der p 1 levels in Dunedin were lower than in Wellington. One reason for this could be the colder winter temperatures in Dunedin affecting house dust mite populations there. In support of this, Der p 1 levels in Christchurch (warmer than Dunedin but colder than Wellington in winter) are intermediate between those from Wellington and Dunedin (79). We also have unpublished data showing a concentration gradient of Der p 1 in New Zealand, with highest levels in the north (Kaitaia), progressively declining towards the south (Invercargill).

A large percentage of Tokelauans now live in New Zealand through migration, particularly following hurricane damage to the Tokelau atolls in 1966. Asthma is rare in Tokelauans having lived all their lives in Tokelau while Tokelauans resident in New Zealand acquire atopic diseases, including asthma, at the same rate as New Zealanders (80). We hypothesised that the low rate of atopic diseases among native Tokelauans may be associated with low indoor allergen levels in Tokelau as the three small atolls sustain little fauna and flora, domestic cats and dogs were eradicated in the 1950s and Tokelauan homes have unglazed windows and uncarpeted floors. We therefore measured indoor floor and bedding allergens (house dust mites, dog, cat, cockroach and endotoxin) in 76 Tokelauan homes and compared these to homes of 30 Tokelauan families resident in Wellington (10). We found that house dust mite allergen levels in Tokelau were over 1000-fold lower compared to New Zealand. Dog and cat allergens were also significantly lower in Tokelau while cockroach allergens were very low in both locations. Thus, Tokelau is a natural low allergen environment at sea level that could explain the low prevalence of asthma and atopy on these atolls. Tokelau provides a unique natural environment to study secondary allergen avoidance among those with established atopic diseases. We are currently studying the effects of 'back migration' of atopic Tokelauans on asthma symptoms and severity.

Intervention studies to reduce house dust mite allergens

As sensitisation to house dust mites is associated with the clinical activity of asthma, it would seem logical that reducing exposure to house dust mite allergens would be of benefit clinically. Many studies have supported this assumption, although some have disputed this. We have conducted four studies looking at ways of reducing house dust mite allergens in the indoor environment. The first study looked

at the effects of frequent carpet vacuum cleaning on house dust mite allergens. In this study nine bed rooms and three hallways in the residents medical officers quarters at Wellington Hospital were vacuumed daily (except weekends) for five weeks (27). Der p 1 levels expressed per unit area decreased on average by 68.5% after five weeks. We went back five weeks after completion of the daily vacuuming phase of the study and collected new dust samples. Der p 1 levels of these samples were higher than those at the start of the study. Thus, daily vacuum cleaning has the potential to significantly reduce house dust mite allergens in carpets, but returning to less regular vacuum cleaning causes this beneficial reduction to be abolished.

One potentially useful method to control house dust mite proliferation and thus allergen production is reduction of relative humidity in the indoor environment. Indeed, a study in Denmark showed that the use of mechanical ventilation and heat recovery was sufficient to significantly reduce absolute humidity and thus reduce or even eliminate house dust mites in mattresses (81). However, outdoor humidity in Scandinavian countries is much lower due to cold and dry winters and houses there are well constructed and air tight. This is not the case in other countries, such as in the UK where a study found no benefit of mechanical home ventilation on house dust mite numbers and allergen levels (82). Given the generally poorly constructed houses in New Zealand and the very high house dust mite allergen loading here, we studied the effects of mechanical ventilation and heat exchange on house dust mite numbers and allergen levels. The study was conducted in Miramar, Wellington where thirty similar homes and their occupants were enrolled (24). The homes were split into three groups. Group A had mechanical ventilation and heat exchange units installed, they were insulated and draught proofed, and electric night store heaters were installed. Group B had identical insulation and draught proofing only, while group C was the control group with no interventions. The homes were monitored regularly for more than a year, and relative humidity, temperature, air exchange, house dust mite numbers and allergen levels recorded. Despite an overall reduction in relative humidity and an increase in temperature in the mechanically ventilated homes, this was not followed by reduction in either house dust mite numbers or in Der p 1 levels. Thus, mechanical ventilation and heat exchange is unlikely to have a significant impact and is not recommended for house dust mite and allergen reduction in New Zealand.

Duvets are widely used as bedding in New Zealand and contain high levels of house dust mite allergens (66). As the thermal death point for the house dust mite, *D. pteronnysinus* is 56°C we wondered whether domestic clothes dryers would be able to reach this temperature and kill house dust mites in duvets. Eight duvets, which had not been washed or dry cleaned for at least six weeks, were studied (34). Live house dust numbers were estimated by a heat exchange method (83) and dust obtained by vacuuming for Der p 1 analysis. The duvets were then individually tumble-dried for one hour with a data logging device recording temperature and relative humidity at eight-second intervals. After tumble-drying, the duvets were again assessed for live house dust mites and Der p 1 levels. Substantial numbers of live house dust mites (mean: 410/m^2) were found in the duvets and these were significantly reduced after tumble-drying (mean: 6/m^2). A mean maximum temperature of 59.3°C was reached during the 1 hr drying period and the mean time to reach the thermal death point was 22 min. House dust mite allergen levels were not significantly changed by the tumble-drying. Thus, tumble-drying with domestic clothes dryers is an easy and effective method of house dust mite reduction. Advice to atopic asthmatics is to first tumble-dry the duvet followed by cold water washing to remove the water-soluble allergens.

Atopic asthmatics are often advised to cover all bedding with moisture permeable, but house dust mite excluding covers as these substantially reduce exposure to house dust mite allergens (84). However, little is known of the effects of occlusive covers on airborne house dust mite allergens. Airborne Der p 1 cannot be quantified using the established double monoclonal antibody ELISA due to their very low levels, however, Japanese researchers have developed a highly sensitive fluorimetric ELISA for air Der p 1 quantification (85). In conjunction with these Japanese researchers we set out to study the effects of occlusive bedding covering on airborne house dust mite allergen levels (28). Mattresses, pillows and duvets of 12 subjects were fitted with occlusive covers and dust samples were collected before, and one week after covering. Air samples were obtained with personal air samplers operating at 1 l/min over seven consecutive nights when the covers were in place, and for a similar period after their removal. We showed that bedding covers significantly reduced house dust mite allergens on each bedding type (5-fold, 16-fold and 76-fold respectively for pillows, duvets and mattresses). The reduction in air Der p 1 levels was more modest, a 6-fold decrease. As airborne Der p 1 may be more relevant for exposure assessment, further studies are required to determine if this modest reduction results in clinical improvement.

House dust mite allergens and allergic diseases

In house dust mite sensitised asthmatics, levels of Der p 1 predict the severity of symptoms (86). However, the link between house dust mite allergen exposure and prevalence of asthma within individual countries is debatable. We undertook a cross-sectional study of seven countries in the Asia-Pacific region (India, Hong Kong, Malaysia, Thailand, Japan, Chile and New Zealand) to determine whether exposure to different levels of house dust mite allergens in these countries was associated with prevalence of atopic diseases and asthma symptoms (87). Mattress and living room floor dust samples were collected from about 36 children in each of 10 centres in these seven countries and analysed for the house dust mite allergens Der p 1 and Der f 1. An ecological analysis was then conducted of allergen levels against asthma symptoms and severity data from the International Study of Asthma and Allergies in Childhood study (88). This study showed that asthma symptom prevalence and having at least four attacks of asthma was associated with house dust mite allergen (Der p 1 + Der f 1) levels. There is a need for further international prospective infant studies in centres with variable house dust mite allergens levels.

We undertook a study to examine the relationship between the indoor environment, atopy and asthma in 233 seven to nine-year old children diagnosed with asthma and 241 control children (89). Living room dust samples were collected for Der p 1 level, indoor exposure information (during the first year of life and currently) collected by questionnaires, and the children were skin prick tested for common indoor and outdoor allergens. Sensitisation to house dust mite allergens was independently associated with current asthma but current Der p 1 levels were not. Furthermore, use of sheepskins and exposure to carpets in the first year of life were also independently associated with current asthma. Thus, exposures in infancy may be more important than current exposure in explaining the prevalence of asthma in childhood. We are currently exploring early life exposures and development of allergic diseases in a multi-centre prospective infant cohort study.

As previously mentioned, there is evidence that both the prevalence and the severity of allergic diseases are increasing (4,5). Strachan

proposed that this may be due to reduced exposure to microbes (90), thus switching the immune system from predominantly Th1 to the allergic Th2 pathway, the so called "hygiene hypothesis (91). Recently, various studies have shown that being born on a farm is protective for the development of allergic diseases (92,93) and this protection seems to be mediated by exposure to higher amounts of endotoxin from Gram-negative bacteria (94). As farming practices differ significantly between New Zealand and Europe, we undertook a study to see if a similar protective effect of farm living was apparent for New Zealand children (95). We studied 494 children living in suburban Dannevirke or on farms in the surrounding region. Allergic symptoms and exposure information was collected by questionnaires, living room floor dust samples were collected for house dust mite allergen and endotoxin levels, and the children were skin prick tested to common allergens. We found that currently living on a farm was associated with a greater prevalence of allergic diseases, but early life exposure to yoghurt and unpasteurized milk consumption, cats, dogs, and pigs was associated with a reduced prevalence of allergic diseases. We also found higher levels of Der p 1, but lower levels of endotoxin on farms. Levels of Der p 1 were not associated with any outcome variable studied.

Not only house dust mite sensitisation, but sensitisation to the cat allergen Fel d 1 is also associated with asthma. Lately, intriguing findings have suggested that early life exposure to cats is associated with a reduced prevalence of atopic sensitisation (96). This is thought to be due to a modified Th2 response where, instead of producing cat-specific IgE when living with cats, children produce increased levels of IgG and IgG4 antibodies to cat allergen (97). We were also interested in seeing whether this was true for New Zealand with its high cat ownership rate and high levels of cat allergen (51). We also were interested in seeing whether a similar tolerance occurs with exposure to high levels of house dust mite allergens. We collected blood, and mattress dust samples from 112 wheezing and 112 control children in a nested case-control study in Hawkes Bay (98). Serum samples were analysed for specific IgE as well as IgG and IgG4 to house dust mite and cat allergens by colleagues in the USA. The dust samples were analysed for house dust mite and cat allergens and endotoxin. As expected, having a resident cat was associated with higher levels of cat allergen in the indoor environment. We found that children who had ever lived with a cat were less likely to have cat-specific IgE, but there was no similar effect on house dust mite-specific IgE. Interestingly, in those sensitised, cat-specific IgE levels were 10-fold lower than house dust mite-specific IgE levels. These results suggest that tolerance to cat allergen is an allergen-specific phenomenon and the strong IgE antibody response to house dust mites could contribute to the high prevalence and severity of asthma in New Zealand.

Conclusions

Levels of house dust mite allergens in New Zealand are high and most likely contributes to the high prevalence and the severity of asthma here. Over the last decade the Wellington Asthma Research Group has focussed on studying the distribution and risk factors for house dust mite allergens, and its association with asthma and allergic diseases. These studies have shown that New Zealand has some of the highest levels of house dust mite allergens in the world with extremely high levels of allergens present in the new born infant's environment; Tokelau is a virtual house dust mite allergen free environment; synthetic beddings contains significantly higher allergen levels than feather beddings due to their permeability to house dust mites; carpets are a significant reservoir for allergens; and domestic clothes dryers can significantly reduce house dust mites from duvets. These studies have contributed significantly to the international literature on house dust mite allergens and have included novel new findings.

Acknowledgements

We wish to thank current and past members of the Wellington Asthma Research Group for their valuable input in our studies. Our studies mentioned in this article have been supported by grants from the Health Research Council of New Zealand, Lotteries Health New Zealand, the Asthma and Respiratory Foundation of New Zealand, the Child Health Foundation of New Zealand, the Wellington Medical Research Foundation, the Marjorie Barclay Trust, and Otago University. We thank the Electricity Corporation of New Zealand for establishment of the allergen laboratory.

This article is dedicated to the late Juliette Lane, a former valued colleague, friend and member of the Wellington Asthma Research Group.

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An unusual case of Septicaemia

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Abstract

We present a case of septicaemia with an initial diagnosis of meningococcal septicaemia, with purpura fulminans, disseminated intravascular coagulation and multi-organ failure. A blood film showed intracellular bacilli and a blood culture finally grew a Gram-negative anaerobic fusiform bacillus, which was subsequently identified as *Capnocytophaga canimorsus*.

Key words: *Capnocytophaga canimorsus*, fusiform bacillus, septicaemia

N Z J Med Lab Sci 2006; 60 (2): 59-60.

Case study

We present a case study of a previously well 60-year-old male, with no known allergies, on no medication. He had until one month previously, smoked 20 cigarettes per day, for 40 years. In November 2002 he was seen at the After Hours Medical Centre. He had been non-specifically unwell for 3 days. The previous night he had suffered rigors. He now presented with sweats, light headedness and chest tightness. He was cyanosed and clammy. A myocardial infarction was suspected and he was immediately transferred to Christchurch Hospital.

On admission, he was cold and clammy with a purpuric rash over his entire body. There were large black areas to his right thigh and left flank. Both ears were black. An initial diagnosis of meningococcal septicaemia, with purpura fulminans, disseminated intravascular coagulation (DIC) and multi-organ failure was made.

Blood tests confirmed part of the diagnosis. Complete blood count (CBC) showed a toxic blood picture with intracellular bacilli and thrombocytopenia. Coagulation showed DIC and biochemistry showed renal failure (Table 1). The blood film, with intracellular bacilli did not confirm the meningococcal disease and was initially thought to be contamination. These bacteria persisted in the blood film for several days (Figure 1). The patient had symptoms of meningococcal septicaemia but blood cultures were negative. Testing for meningococcal DNA was negative.

The bacteria were fastidious and did not show a positive blood culture for three days. It finally grew a Gram-negative anaerobic fusiform bacillus. This in itself proved difficult to identify and was sent to ESR for identification.

Treatment for this severely ill man was antibiotics, Activated Protein C with fresh frozen plasma and platelet support for the DIC, dialysis for renal failure, and intubation for respiratory failure. Over the next two weeks his condition remained poor. He had large blisters over his body and his feet were necrotic. He had continuing renal failure and respiratory distress. A decision was made to perform bilateral below knee amputations. After surgery he recovered quickly and two weeks later he was discharged home. During his recovery phase the identification of the Gram-negative bacillus came back from ESR. It was *Capnocytophaga canimorsus*. The source of his infection was never isolated as he had no known contact with cats or dogs.

Table 1. Blood results

CBC		Ref range	Coagulation		Ref range
Hb	179g/L	135-175	INR	4.3	0.9-1.2
Hct	0.52		APTT	>150sec	23-36
MCV	98fl	80-100	Thrombin Time	44sec	18-25
WBC	$4.3x10^9$/L	4.0-11.0	Fibrinogen	1.0g/L	1.5-4
Neut	$4.1x10^9$/L	2.0-8.0	D Dimer Assay	>1000	<250
Lymph	$0.3x10^9$/L	1.0-4.0			
Platelets	$11x10^9$/L	150-450	**Biochemistry**		
Film Comment:			Urea	10.8mmol/L	2.7-7.8
Neutrophils show marked toxic changes.			Creatinine	0.25	0.05-0.11
Neutrophils show presence of intracellular bacteria.			AST	129U/L	10-50

Discussion

Capnocytophaga canimorsus, previously known as dysgonic fermenters-2 (DF2)(1), is a fastidious, thin Gram-negative rod that is rarely pathogenic in humans. It grows best on chocolate agar in 5% carbon dioxide and it may take up to 7 days to see growth on the plate (4). Blood cultures are usually positive in 6 days but there have been some cases where blood culture has not become positive until 14 days (5). *Capnocytophaga canimorsus* is normal oral flora of cat and dog saliva, canimorsus is Latin for dog bite (1). Bacteria that are normal flora in the mouths of cats and dogs do not usually infect humans. In the rare event of this happening, it can lead to catastrophic illness. The bacteria are aptly named since humans are infected following dog and occasionally cat bites or licking of open wounds or sores. It has been isolated in about 16% of dogs and 18% of cats (1,2). However, about 20% of patients have had no known contact with animals as was the case in this study.

Capnocytophaga canimorsus was first recognized as a cause of sepsis in 1976. Subsequently, there have been more than 150 cases recognized world wide, mainly occurring in adult men (1,3). Risk factors for developing severe sepsis include splenectomy, alcohol abuse and heart disease. Two third of cases were in patients who were immunosuppressed.

Five to six days after a bite, a scratch, or other exposure, clinical symptoms usually begin. Patients typically present with fever, vomiting and abdominal pain. Shock, renal failure and adult respiratory distress syndrome may rapidly follow. DIC and purpura fulminans occur in 36% of cases and can result in gangrene, requiring amputation. Gangrene may also develop at the site of the bite. Mortality with septicaemia is around 30% (1).

Specific identification of *Capnocytophaga canimorsus* may be obtained rapidly by microscope examination of the blood film. The thin Gram-negative bacilli with curved tapered ends can usually be seen intracellularly. The blood film is more likely to show the presence of the bacteria in asplenic patients or those with a high bacterial burden. It is positive in about 90% of cases (1). Blood cultures usually become positive within six days, but may take up to 14 days.

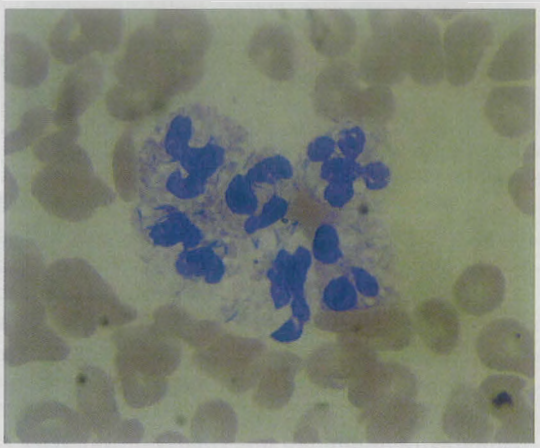

Figure 1. Intracellular *Capnocytophaga canimorsus*

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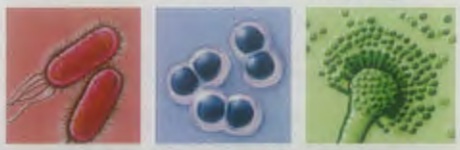

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ACID-BASE METABOLISM
Practical Details of the Astrup Macro Method of Assessment

H.G. BLOORE, B.Sc

(Clinical Laboratory, Wairau Hospital, Blenheim)

The Astrup method of assessment of changes in the acid-base state of patients is a quick, reliable procedure, giving positive information of an unequivocal character.

It involves obtaining the following values:-

(1) Actual pH of whole blood, anaerobic ally collected.

(2) Whole blood pH, determined after equilibrating the sample with a carbon dioxide-oxygen mixture of known CO_2 tension.

From the latter value (equilibrated pH) plus the haemoglobin value, by using a nomogram, may be ascertained the following:-

Base Excess or Deficit, Standard Bicarbonate and Buffer Base.

If in addition, the former value (Actual pH) is obtained, then the following may also be ascertained:-

pCO_2, Total CO_2, Actual Bicarbonate, and CO_2 combining power.

Of these, the important values are-

(1) Base Excess or Deficit - reflecting metabolic changes.

(2) pCO_2 - reflecting respiratory changes.

Since metabolic changes in the acid-base state are by far the commonest in general hospital work, it is found that most requirements are met by simply measuring the pH of the equilibrated sample of blood, estimating its haemoglobin level and referring to a nomogram.

Definitions as given by Astrup et al.

(1) *Standard Bicarbonate e* (6,7).

This is the bicarbonate content of the plasma part of whole blood measured at 38°C, at a pCO_2 of 40mm. Of Hg, with the haemoglobin fully oxygenated.

Under these standardised conditions, the pH of the sample of blood is a direct indication of its bicarbonate content. The conversion of pH to standard bicarbonate is obtained either from the calibrated scale of the pH meter, the Henderson-Hasselbach equation, or from the nomogram of Anderson & Engel (1). Expressed in m.Eq/litre.

(2) *Buffer Base* (1,8) (B.B.).

The sum of the buffer anions, mainly bicarbonate and proteinate ions-in m.Eq/litre.

(3) *Base Excess* (B.E.).

The difference between the Buffer Base found and the normal buffer base. Expressed as plus or minus (the latter indicating a deficit) in m.Eq/litre.

(4) pCO_2.

This is the partial pressure of CO_2 in the blood at the moment of sampling. This is the measure of the respiratory component of the acid-base state, expressed in m.m. of Hg.

(5) *Normal Buffer Base* (1) (NBB).

The Buffer Base of blood with a pH of 7.38 at a pCO_2 of 40 m.m. Hg.

NBB==40.8 + 0.36x haemoglobin conc. (in g.per 100 ml.).

As the standard bicarbonate does not give directly the amount in m.Eq/litre of fixed acid or base causing a change in the base content of the blood, the value of Base Excess is employed instead.

PRINCIPLE OF THE ASTRUP METHOD

If a graph is prepared on semi-log paper (logarithmic, 1 cycle x natural), showing the relations between pH and log pCO_2 of a blood sample, the result is a straight line. The slope of the line is a function of the buffer capacity of the blood, and the position of the line is a function of the base content of the blood.

Once the line is obtained in any case, if the actual whole-blood pH is found, then the actual pCO_2 can also be found. The nomogram of Anderson and Engel is such a graph (1).

The original Astrup apparatus employs equilibration with one gas mixture and requires a knowledge of the haemoglobin

level, while the new Ultra Micro Astrup Method employs equilibration with two samples and two different gas mixtures, and the haemoglobin value is not required.

I propose to outline the actual method of using the original Astrup apparatus, as employed in this laboratory.

APPARATUS

Astrup Macro Apparatus Type E50101 - made by Messrs Radiometer, Copenhagen, kept permanently warm via the water jacket at 38°C.

Water Bath - a plastic tank is suitable and cheap.

Thermostatted circulating pump - The Techne Tempunit is satisfactory.

Ph Meter - a reading accuracy of 0.005 pH unit is desirable. The combined electrode of the Radiometer Model 22 pH meter is designed to fit the Astrup apparatus.

The Radiometer extension meter gives the required reading accuracy.

CO_2/O_2 *Cylinders* - ordered from N.Z. Industrial Gases Ltd., to contain 5.6% CO_2 and 94.4% oxygen. The CO_2 content must be known to within 0.05%, i.e., to two significant figures, and should be within the range of 5.4% to 5.8% CO_2.

These mixtures are analysed by the Dominion Laboratory before being forwarded.

Reducing Valve - British Oxygen Co's gas regulator valve is suitable.

Stoppered Tubes - for distilled water and standard buffer, filled and placed in rack in water bath at 38°C., five minutes before use.

Syringes - 10 ml., oiled and dry-heat sterilized in some sort of container to maintain sterility.

REAGENTS

1. *Heparin Solution* - 5000 units/ml.
   For adding to syringe
   Dissolve 200 mgms (== 10,000 units) of calcium heparin in 2 mls. Sterile distilled water, in sterile dropping bottle.
   Store in refrigerator.
   Use 1 drop per syringe.
2. *Heparin - Fluoride Solution*
   For Base Excess Bottle:
   Sodium heparin 10,000 units or 200 mgms. Powder
   Sodium Fluoride A.R. 2.5 grams
   Distilled water to 100 ml
   Place 0.5 mls. Of this solution (1 mgms. Or 50 units of heparin and 12.5 mgms. NaF) in bottle and dry in oven. For 5 mls. Blood.
4. *Silicone Defoamer* as purchased from Messrs Radiometer.
5. *Potassium Chloride A.R.* as fine crystals and as saturated solution.
6. *Standard Buffer Solutions.....*

A. M/15 primary potassium phosphate
   2.2695 gms of dry KH_2PO_4 (Sorenson salt, Merck) are dissolved in 250 mls. of deionized water.
   Refrigerate.

B. M/15 Secondary Sodium Phosphate-
   11.8667 grams of $Na_2HPO_4 . 2H_2O$ (Sorenson in 1000 mls. Of deionized distilled water salt, Merck) are dissolved.
   Refrigerate.
   Working Solution M/15 pH 7.380 ± 0.005 @ 38°C.
   Warm stock solutions to room temperature and mix 20 mls. of A with 80 mls. of B.

7. *Paraffin Oil/Ether* - equal parts neutralized paraffin oil and anaesthetic ether.
   The oil may be neutralized as follows:-
   In a 500 ml. separatory funnel place about 200 ml. of oil, an equal volume of distilled water, and a few drops of 0.1% phenol red solution.
   Add 0.02 N NaOH a drop at a time with vigorous shaking until the water solution of indicator becomes permanently pink.
   Centrifuge the oil to remove suspended water droplets.
   Decant the clear oil with care, and store in a stoppered bottle.
8. *Deionised Water*
   Distilled water is passed through a column of ion exchange resin. The resin employed is Biodeminrolit supplied by J. J. Niven & Co. Ltd.
   This process is quick, and removes CO_2 from the water without the need to boil it.
   This water is hereinafter referred to as Water.

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PROCEDURE

Immediately prior to performing an estimation, the pH meter is switched on to warm up, and adjusted to read the correct pH when the chamber of the Astrup apparatus is filled with standard buffer solution at 38°C.

After rinsing the chamber three times with water at 38°C, the instrument is left with the chamber filled with water, and is ready for use.

COLLECTION OF BLOOD

(1) *When Base Excess or Standard Bicarbonate* only are required:-

Venous blood is collected with any dry syringe without any particular precautions, except avoiding venous stasis as far as possible, and 4-5 mls. are placed in a specimen bottle containing dried heparin/sodium fluoride mixture, and shaken briefly. This specimen is suitable for analysis for an hour or two.

(2) *When actual pH*, in addition to Base Excess and Standard Bicarbonate is required:-

Venous blood from a preferably warmed arm is collected with preferably no stasis, in a well-fitting, oiled 10 ml. syringe to which has been added just before using one drop of a calcium heparin solution (5,000 I.U. per ml.).

This drop, plus the oil, leaves almost no air space in the syringe, thus assisting the anaerobic collection of the sample.

Immediately on removing the needle from the vein, the syringe is pointed upwards, and any tiny air bubble is ejected into a cotton wool swab held over the needle point. A large drop of mercury is then sucked into the syringe from a small bottle containing 1-2 mls. The needle point is then pushed into an ordinary clean cork and the blood mixed thoroughly.

The actual pH should be determined as soon as possible.

Some 12-13 mls. of blood can be collected in a good 10 ml. syringe, and this provides sufficient specimen for actual pH, Base Excess or Standard HCO_3, Hb and P.C.V. and Na, K, Cl and N.P.N. estimations. (For the N.P.N. a small correction must be made, allowing for the nitrogen in the heparin. This may readily be found by experiment).

Just before the pH determination, the syringe is briefly warmed in hot water.

DETERMINATION OF ACTUAL BLOOD pH

The water is ejected from the chamber of the apparatus and mercury raised up to fill the bottom of the cup, which is then dried with a piece of folded filter paper. The specimen is thoroughly mixed in the syringe, the needle removed and replaced with a 1 inch piece of sphygmomanometer tubing (narrow bore and thick wall) which is immediately filled with blood.

As rapidly as possible, the syringe is inverted and the end of the tubing pressed firmly against the bottom of the cup, under the surface of the mercury.

By manipulating the taps, the mercury in the chamber is run down, drawing blood after it, until the chamber contains 2-3 mls. of blood-enough to cover the electrode. Both top and bottom taps are closed, the syringe removed and the needle and cork replaced on it. The pH meter is switched to the reading position and a reading made after about 1 minute.

The blood may then be raised into the cup and pipetted of for other tests, e.g., Hb and P.C.V., and N.P.N.

After rinsing the chamber 3-4 times with warm water, the pH meter setting is checked again with warm standard buffer. If necessary, the process must be repeated with more blood from the syringe, which may be kept in the water bath at 38°C. in the meantime. The chamber is finally left filled with water.

The remaining blood in the syringe is now distributed into specimen bottles as required, e.g., 3 mls. in Base Excess bottle, and 6-7 mls. in a centrifuge tube for plasma Na, K and Cl. estimations, if these are requested.

DETERMINATION OF EQUILLIBRATED pH

The pH meter and apparatus are prepared as described above to the point where the mercury fills the lower part of the cup and the latter is dried.

About 2.5 mls. of blood from the 'Base Excess' bottle is poured into the cup, and a small portion of antifoam emulsion is smeared around the stem of the cup with a piece of glass rod.

Mercury and blood are then run slowly into the chamber, at the same time using the glass rod to help some of the antifoam emulsion to pass into the chamber also. This emulsion is like vaseline in consistency and very little is actually needed to prevent foaming and consequent haemolysis.

When the top of the mercury column is level with the lower edge of the side tube of the chamber, the lower tap is closed.

With the tap of the side arm closed, and the flow indicator tap turned to air, the gas cylinder tap is turned on, and by adjusting the reducing valve control, 3-4 bubbles per second are allowed to flow through the indicator. By turning the above-mentioned taps, the gas is caused to flow through the blood sample, and any necessary adjustment is made to the flow rate. If flow is to fast, blood is splashed about vigorously and haemolysis may occur, while if the rate is to slow, equilibration will not be complete in the desired time.

After about 2 minutes, the main cylinder valve may be closed, the reducing valve containing sufficient gas to last the

remaining three minutes. The life of the cylinder may thus be almost doubled (if the cylinder is nearly empty, the reducing valve may hold perhaps only 1 minute's supply of gas, and the final portion is sprayed all over the room!)

After a total of 5 minutes' bubbling, proceed as follows:-

Turn gas supply to flow to air from flow indicator, raise the blood to cover the electrode, close top and bottom chamber taps, switch pH meter to reading position, turn of gas at cylinder reducing valve, and read the pH after about half a minute.

The extension pH meter made by Radiometer is calibrated also in m. Mols. per litre of standard bicarbonate, and this may be read of directly, provided the pCO_2 of the cylinder gas is 40 mm. Hg. or close to it.

The blood is then pipetted off and the pH meter buffer again checked. If necessary, the equilibrated pH may be determined again on the same blood sample. The chamber is left filled with water and the pH meter switched off. As the whole apparatus is kept constantly at 38°C. it is always ready for use.

CORRECTIONS

1. The pCO_2 of the cylinder gas is found according to the following (3):-

$$pCO_2 == \frac{(B-W) \times \text{per cent } CO_2}{100}$$

where B == barometric pressure in m.m. Hg., and W is the vapour pressure of water at 38°C. (==50 m.m. Hg.).

2. If the pCO_2 thus found deviates from 40 m.m. Hg., and is between 36 and 44 mm. Hg., it may be convenient, in using the nomogram, to correct the equilibrated pH as follows (6) :-
   PH_{40} == pH measured + 0.006 x (pCO2 used - 40)
   It is helpful to prepare tables in each case for quick reference. A table should also be prepared relating Normal Buffer Base and haemoglobin concentrations at 0.5 grams per cent intervals.

USING THE NOMOGRAM OF ANDERSON AND ENGEL

In the procedure described, when equilibration is carried out with a single CO_2/O_2 mixture, the Haemoglobin value is required to obtain the Normal Buffer Base of the sample. We thus have the following items for use with the nomogram:-

Normal Buffer Base, Actual pH, and Equilibrated pH.

The Normal Buffer Base gives the slope of the line to be drawn, the equilibrated pH gives the position of the line, whence the Base Excess may be directly obtained, and the point on the line provided by the Actual pH gives the pCO_2 of the sample.

When the Ultra Micro Astrup method (2) of analysis is performed, equilibration is carried out at two different CO_2 tensions and the position and slope of the line obtained, without the need for knowledge of the haemoglobin value. This method employs capillary blood, is quicker to perform than the original macro method described here and is thus eminently suitable for serial determinations, especially in following the respiratory component of the acid base metabolism.

In practice, using the macro method the whole procedure of obtaining actual pH and Standard Bicarbonate and Base Excess figures takes 12-13 minutes total elapsed time. If Standard Bicarbonate or Base Excess only are required, as is usually the case here, only about 5 minutes' work is involved. During equilibration other work may be proceeded with.

The appended references cover the work which has been done in the course of the development of this new method of investigation of acid-base state up to the present time.

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[Reprinted from the Journal of the New Zealand Association of Bacteriologists, 1947; Vol.1, No.4]

COUNCIL MEETING, NOVEMBER 1946

A Council Meeting was held in the rooms of Dr. P. P. Lynch, Kelvin Chambers, The Terrace, Wellington, on Saturday, November 30th, at 10 a.m.

There were present Mr. E. L. F. Buxton (Chairman) and Messr. N. I. Ellison, J. I. G. Peddie, D. H. Adamson, G. W. McInley and D. Whillans. The Chairman opened by congratulating the Council Members on their fine effort in attending the meeting in view of the long travelling time involved in most cases.

After a number of routine matters had been dealt with the following members were elected to the Association:

Senior - Mr. M. Morris, c/o Medical School, Dunedin.

Junior - Miss B. Broughton and Mr. A. Harper, Kew Hospital, Invercargill.
Mr. G. B. Kiddle, c/o Dr. P. P. Lynch, Wellington.

Mr. D. H. Adamson, convenor of the Committee set up at the last Annual Conference on Salaries, presented the report of his committee. After a prolonged discussion it was decided to write to the Director-General of Health and the Secretary of the Hospital Boards' Association, copies being sent to the Directors of all Clinical Laboratories. The wording was thus:-

"In view of the increasingly high standard of work being demanded of Professional Staffs in Clinical Laboratories in New Zealand, and in consideration of the increased cost of living, my Association submits for your consideration and approval the following salary scale:

Cadets -
First year, £130.
Second year, £170.
Third year, £270.
Cadets living away from home to receive a boarding allowance of £78 during the first two years.

Trainees -
First year, £295.
Second year, £320.

Qualified Bacteriologists -
(a) On qualifying, £450, rising by £50 p.a. to £600.
(b) If in charge of a department during this time, £50 extra.
(c) Bacteriologists in Laboratories not directly controlled by resident Pathologist and Senior Supervisors in Laboratories controlled by a resident Pathologist.
Minimum £650 to Minimum £750, to rise from the lower to the higher rate in two years. Thereafter the salary to be increased in accordance with the importance of the position held.

My Association considers that such a scale is necessary to encourage the entrance of suitable candidates into, and the retention of those already engaged, in the Profession."

The report from the Committee on the Syllabus of Training for Hospital Bacteriologists was then presented by Mr. N. I. Ellison, convenor, and discussed at length by the Council. It was decided to write to the Director-General of Health in the following terms, copies being sent to the Secretary of the Hospital Boards' Association:-

"My Association feels that the Syllabus of training for Hospital Bacteriologists at present in force does not cover the scope of work required and submits the following Syllabus for your consideration and approval.

SUGGESTED SYLLABUS OF TRAINING FOR HOSPITAL BACTERIOLOGISTS FOR THE CERTIFICATE IN BACTERIOLOGY AND CLINICAL PATHOLOGY

1. **The Preparation, Operation and Maintenance of Laboratory Equipment:**
   Microscope, including dark ground condenser and micrometers; colorimeter; hydrogen ion and photoelectric apparatus; microtome; incubators; autoclaves; thermo-regulated apparatus; stills; filters; anaerobic equipment; laboratory requisites.
2. **The Preparation of Reagents, Etc.:**
   Stains; reagents; normal; standard; molar; and buffer solutions; culture media; preparation of parenteral solutions.
3. **Bacteriology:**
   General principles of bacteriology and epidemiology; the systemic study of micro-organisms and some knowledge of viruses, particularly the pathogens of men; their biological classification, morphology, physiology, metabolism and nutrition. The isolation of micro-organisms in pure culture and their identification. The bacteriological analysis of air, foods, water and soil.
4. **Immunity:**
   General principles of immunity and serology. The function of antigens, antibodies, complement, agglutinins, opsonins and precipitins. Widal reaction. Anaphylaxis. The production of immune serum, determination of titre and absorption of antigenic factors. Specific and non-specific antigens. The preparation of reagents for the Wasserman and Kahn tests. The preparation and use of vaccines and fluids for skin sensitivity tests.
5. **Antibiotics:**
   The dispensing, storage and distribution of antibiotics and their methods of assay. Determination of the resistance of organisms to penicillin and to the sulphonamides. Standard technique for the testing of disinfectants.
6. **Haematology:**
   Collection of specimens; origin and development of cells; enumeration of erythrocytes, leucocytes, platelets and reticulocytes; examination of stained films (including bone marrow), differential counts and blood parasites. The estimation of haemoglobin, sedimentation rate, packed cell volume, and blood indices. Fragility test; coagulation, bleeding and prothrombin times. Heterophile antibody tests. Blood grouping, Rh factor and blood bank.
7. **Parasitology:**
   A knowledge of the common parasites infesting men, their identification and transmission.
8. **Examination of Body Fluids and Excreta:**
   Cytology, bacteriology and chemistry, where applicable, of puncture fluids, pus, sputum and excreta.
9. **Biochemistry:**
   Qualitative tests and quantitative estimations where relevant for the following:-
   (a) Blood
   Sugar, T.N.P.N., urea, uric acid, calcium, phosphorus, phosphatase, chloride, protein, bile, alkali reserve.
   (b) Cerebrospinal Fluid
   Sugar, T.N.P.N., chlorides, protein, colloid gold.
   (c) Urine
   Reducing substances, ketone, chloride, bile and its derivatives, Urea, albumin, blood pigments.
   (d) Faeces
   Occult blood; fat and fatty acids.
   Tests for renal efficiency, sugar tolerance, liver and gastric function, and enzyme activity. Analysis of calculi.
10. **Histology:**
    The routine preparation of tissues for histological examination and the preparation and mounting of museum specimens.
11. **Laboratory Animals:**
    A practical knowledge of the inoculation, examination, propagation and feeding of laboratory animals.

The Secretary then read the report from the Committee on the proposed Intermediate examination to be held at the end of three years training. (Mr. I. W. Saunders, Convenor.)

The syllabus for this examination was discussed at length, but was finally referred back to the original Committee with the suggestion that it be brought into line with the proposed Syllabus of Training of Hospital Bacteriologists. (It is suggested that this examination be open to all training under a qualified Bacteriologist or under a Pathologist, the aim being to stimulate junior workers in both types of laboratory as well as to provide opportunities which are now completely lacking for junior workers in laboratories under the control of a Hospital Bacteriologist. It will be noticed that it is proposed to

increase the period of Cadetship to three years and cut the Traineeship to two years. It is further recommended that, in making appointments to Training Laboratories, preference should be given to those who have completed their third year and passed their Intermediate examination in laboratories under the control of a Hospital Bacteriologist.)

The question of Prize essay was then dealt with, and the Council allocated the sum of £2/2/- to be competed for by Junior members of the Association under the following conditions:-

(1) The Essay shall be the unaided work of a member who is a Junior member of the Association at the time of submitting the Essay.
(2) The Essay shall be in the hands of the Secretary of the Association not later than May 31st, 1947.
(3) The Essay shall be unsigned, but shall be accompanied by an enclosure giving the name and address of the entrant.
(4) The value of the prize for the 1947 competition shall be £2/2/- and shall be presented, together with an appropriate Certificate, at the third Annual Conference of the Association, 1947.
(5) The decision of the Judge shall be final.

Conference, 1947:

The tentative date for the Conference, 1947, was fixed for Friday and Saturday, July 18th and 19th, 1947, and the place as Christchurch. It was stated that the preliminary work for a satisfactory Conference was well in hand.

The meeting closed at 5.30 p.m.

[Reprinted from the Journal of the New Zealand Association of Bacteriologists, October 1949; Vol.4, No.4, Page 41]

EDITORIAL

Editors:- D. Whillans, Auckland,
H.T.G. Olive, Wellington and M.O.Ekdahl, New Plymouth

Not for the first time does it become necessary to remind members of the Association that a Journal requires more than an Editor, a Printer and a Publisher. There must be Contributors, without whom the Editor, be he ever so resourceful, must finally give up in despair.

Your Editor, by giving up his own hobby and devoting his spare time to all offices in the production of the Journal, has managed to struggle as far as the end of Volume 4, but if more help in the way of contributions is not forthcoming, must inevitably consider the game not worth the candle.

At each Conference he is heartened by the flattering references to his ability and overwhelmed by offers of assistance; unfortunately, these are mere words and are rarely followed by articles.

Writing a clear concise article requires much time and the elimination of much muddled thinking. In your Editor's experience at least five re-writes are necessary before an article is ready for publication. Recently it cost him eleven re-writes before an article was deemed ready for submission to a certain Journal.

Ask yourself why YOU have not yet written an article, and set out forthwith to remedy the deficiency.

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Auckland University of Technology - NZIMLS prize for 2005

JULIAN VERRAN

Julian was born in Levin in 1976. His schooling was in Hamilton, with a 5 year stint in Papua New Guinea whilst his family relocated there for work. Julian completed a BSc at Waikato University and then worked for 1 year in Chemistry for Hills Laboratories in Hamilton. He then headed off for his OE for 3 years, where he worked in and around London in the National Blood Service in Tooting, in Chelsea Hospital Blood Bank, and in Kings College Hospital in the TB unit.

He completed his BMLS at AUT in 2005 and is currently working in the Processing Department at New Zealand Blood Service, Auckland. He will soon be starting in the Cryogenics lab, learning to process bone marrow donations. Julian enjoys learning new things and likes new challenges. In his spare time he writes electronic breakbeat music. In addition to the NZIMLS prize for the top graduating BMLS student, Julian also wins the top graduating student in Haematology and the top graduating student in Transfusion Science prizes (all to be awarded at an AUT ceremony in May).

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.

THE Pacific WAY

News from the PPTC

Annual Meeting

The Annual Meeting of the PPTC was held on the 19 May 2006 at the Centre located on the Wellington Hospital Campus. The following is an extract from the Annual Report presented by the Chairman, Dr Ron Mackenzie.

Chairman's Perspective

It is with pleasure that I introduce the PPTC Annual Report for 2005. During this year the Centre has performed well in its three core activities of providing teaching, external quality assurance programmes and Pacific regional consultancies, all these activities are reviewed in the pages which follow.

Of special significance in 2005 was the PPTC Silver Jubilee celebration. This was an occasion which gave opportunity to acknowledge the many people and organizations that have given support and encouragement over the years.

While looking back during this Jubilee year, 2005 was also a time for looking forward and much work was carried out in setting new strategic directions for the Centre. Notably amongst these was the continuation and expansion of the PPTC distance learning programme in medical laboratory sciences through the WHO distance learning website, Pacific Open Learning Health Net (POLHN).

The second major event which took place in 2005 was the long awaited review of the PPTC by NZAID. The review validated the work of the Centre and concluded that the PPTC programme fitted in well with the NZAID Pacific strategy. The 2005 review further strengthened the good working relationship which exists between NZAID and the PPTC which began more than 25 years ago with the External Aid Division of the Department of External Affairs.

The place of the PPTC as a teaching resource for medical laboratory sciences was further enhanced during the year when the Centre was contracted by the Global Fund to fight AIDS, TB and malaria (GFATM) to provide in-country training. This was undertaken by the Director and resulted in over 40 laboratory technicians being trained in laboratory diagnostic methods for STI including HIV. Arising from this, a number of new training opportunities are being explored.

2005 also saw a continuation of the regional quality assurance programme to 27 hospital laboratories in the Pacific and Asian regions. Our thanks go to the programme co-ordinators for their contribution and to Christine Story for the smooth running of this programme.

Finally I would like to express my thanks to the Board members, the voluntary tutors and the Director and staff who together made 2005 another vintage year for the Pacific Paramedical Training Centre.

Blood cell morphology course April - May 2006

A five week blood cell morphology course was held during April and May at the PPTC. There were two participants on the course which was tutored by Marilyn Eales. Doris Manongi from the Honiara National Referral Hospital in the Solomon Islands and Wati Vukialau from Ba Mission Hospital in Fiji were able to update their blood cell morphology knowledge. Doris and Wati also toured Wellington Hospital Laboratory and Wellington Medical Laboratory which were of great interest to them comparing the workload and technology from their home laboratories. At the end of the course a certificate presentation was held at the Centre where Philip Wakem, PTA Haematology Section, Laboratory Services, Wellington Hospital presented certificates to Doris and Wati.

Blood Cell Morphology Course
Back Row: Doris Manongi, John Elliot, Wati Ukialau
Front Row: Christine Story & Marilyn Eales

Laboratory management and quality systems training held in Qui Nhon, Vietnam

In March 2005 three staff from the Health Services in Qui Nhon, Binh Dinh Province, Vietnam were sponsored by the NZ Vietnam Health Trust [NZVNHT] to attend a course on laboratory management and quality systems conducted at the PPTC, Wellington. This course is based around the introduction and implementation of ISO15189. Following their return to Vietnam, they developed a five year plan for the implementation of ISO 15189 into medical laboratories in the Binh Dinh Province in conjunction with the NZVNHT.

Participants in the first course

A group of participants in the 2nd course

As a result, late in 2005 the PPTC was invited to conduct courses for medical laboratory staff on the basics of ISO15189 and how this standard could be implemented into medical laboratories in the Binh Dinh Province and in March this year this training commenced. Initially Phil Barnes of IANZ conducted a workshop for senior health administrators from all the hospitals in the Province explaining to them the importance and relevance of ISO15189. Following on from this John Elliot, PPTC Director, conducted 2 one week workshops which were attended by about 60 senior laboratory staff from all the laboratories in the Province. During these workshops the basic concepts and requirements of the standard were introduced and examples of a quality manual and standard operating procedures [SOPs] were discussed. It was great to see the enthusiasm of both laboratory staff and the Department of Health for the introduction of standards and hence the continuing improvement in quality.

News from the coconut grapevine

We recently received information that Dr V F Asaua had passed away quite suddenly. Dr Asaua had been the Pathologist at the National Hospital Laboratory in Apia, Samoa for a number of years. We extend our sympathy to Vaomalo Kini and the staff of the laboratory.

We also heard that Faiatea Latasi [Fab] has retired from his position as charge technologist of the laboratory in the Princess Margaret Hospital, Tuvalu. We wish Fab a long and enjoyable retirement.

HSIG questionnaire

Haematology
Special Interest Group

Journal article questionnaire for the Haematology Special Interest Group

Blood film examination for vacuolated lymphocytes in the diagnosis of metabolic disorders; retrospective experience of >2500 cases from a single centre.
Journal of Clinical Pathology 2005; 58:1304-1310.

Questions
True or False (Questions 1-6)

1. In blood film monocytes can be confused with lymphocytes because monocytes may show cytoplasmic vacuolation.
2. Fresh EDTA or heparinised anticoagulated sample is suitable for blood film examination to look for vacuolated lymphocytes.
3. The technique of blood film examination for vacuolated lymphocytes requires examination on thin end of the film (near the tail) than the thicker area.
4. A range of metabolic disorders resulting in abnormal accumulation of metabolic products may exhibit abnormal cytoplasmic vacuolation of lymphocytes.
5. In this study the most common clinical indicator was ophthalmic indication having the highest frequency of vacuolated lymphocytes.
6. Many metabolic disorders are increasingly being associated with specific gene defects which may be identified with molecular diagnostic techniques.
7. How many lymphocytes should be examined in a standard film?
8. List 2 tests used in this study to confirm the diagnosis of a metabolic disorder.
9. The clinical features in conjunction with characteristic of lymphocyte vacuolation are highly suggestive of which metabolic disorder?
10. Name 2 tests to be the "gold standard" tests for diagnosis of specific metabolic disorders?

Answers on page 84

Prepared by Kamla Prasad, Microscopy Technical Specialist, Haematology lab, Lab Plus, Auckland Central Hospital
Email: kamlap@adhb.govt.nz or Ph 3074949 Ext 7580

New Zealand Journal of
Medical
Laboratory
Science

60 Years of Continuous Publication Journal Prize

In 2006 the Journal will celebrate 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. Time is running out, to possibly make the November issue, submissions must be with the Editor no later than by the end of August to allow time for peer-review. Please feel free to contact the Editor, Deputy Editor or Members of the Editorial Board if you want advise or guidance.

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.

Immunology
Special Interest Group 2006
Dunedin
Saturday 30th September

Please send your expression of interest in giving a paper, title and general description, to: helen.vanderloo@sclabs.co.nz. Registration information and further details to follow at: www.nzimls.org.nz

Journal-based questionnaire

We received a very good response for the inaugural journal-based questionnaire in the April 2006 issue of the Journal with 402 members submitting answers. All passed (at least 7 out of 10) with nearly 50 % attaining 10 out of 10. All have earned 5 CPD points to be claimed under section 18 of the CPD points table.

Below are the questions from the April 2006 issue together with the correct answers.

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

In the final phase of myelofibrosis, a progressive decline of the WBC can be seen.
False. A progressive rise of the WBC can be seen with counts of 100-200 x 109 possible.

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

Conventional drug therapies for MMM are largely palliative.
True.

In 1973 colour advertisements were published in the Journal for the first time.
False. 1983, not 1973.

To celebrate 60 years of the Journal, a special prize of $500 is offered for the best case study published during 2006.
True. Still time to submit case studies for this prize!

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.

Porphyrins have characteristic electronic absorption spectra with an intense maximum around 500 nm that is known as the Soret peak.
False. Around 400 nm, not around 500 nm.

Raised screening test results are considered diagnostic of porphyria.
False. Raised screening tests must not be considered diagnostic of porphyria.

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

Journal-based questionnaire for this (August 2006) issue

Below are 10 questions based on this issue of the Journal. The answers can be found anywhere in the issue (articles, editorials, adverts, letters), thus read the entire Journal. The questions are in the format of True/False.

Answers are to be submitted through the NZIMLS web site (www.nzimls.org.nz) only, no mail or fax submissions will be considered. When in the NZIMLS web site, go to Journal and in there go to Journal-Based Questionnaire.

The site for submitting your answers will remain open until 5pm on Thursday 31 August 2006 after which it will close. 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.

1. The NZIMLS provides the CPD programme, not the MLSB
True **False**

2. The NZIMLS issues the Annual Practicing Certificate
True **False**

3. House dust mites belong to the class Arachnida.
True **False**

4. *Blomia tropicalis* is the dominant house dust nite species in New Zealand
True **False**

5. Double-monoclonal antibody ELISA remains the gold standard for measuring Der p 1.
True **False**

6. Synthetic pillows contain significantly higher levels of house dust mite allergens than feather pillows.
True **False**

7. House dust mite allergens are present in high quantities in human hair.
True **False**

8. Increasing exposure to microbes switches the immune system from the predominantly Th2 to the allergic Th1 pathway.
True **False**

9. In the Historical Article, using the macro method the whole procedure of obtaining actual pH and Standard Bicarbonate and Base Excess takes 12-13 minutes.
True **False**

10. *Capnocytophaga canimorsus* is a fastidious, thin Gram-negative rod.
True **False**

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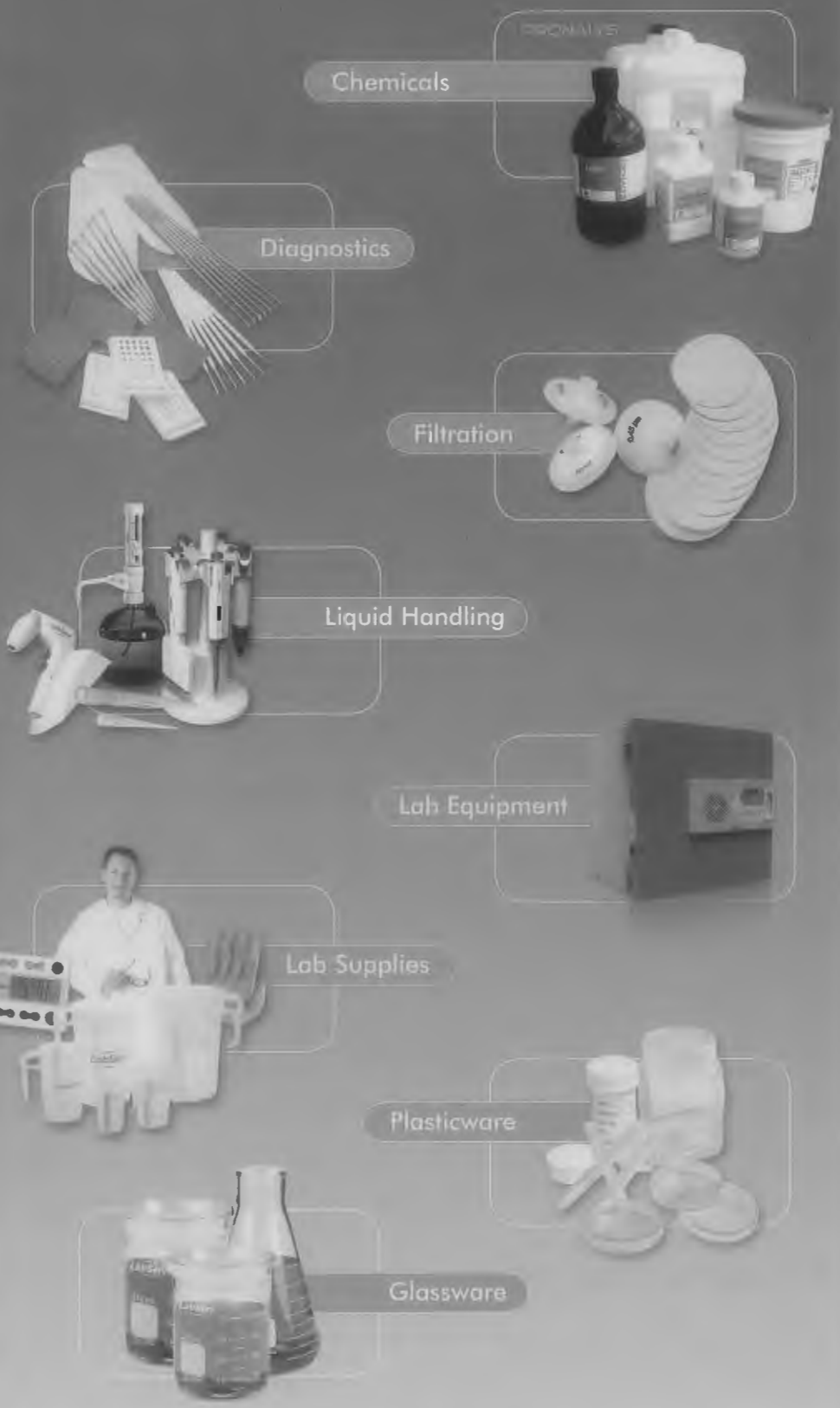

Biolab: Stand 20

Come and visit our stand at the
NZ Institute of Medical Laboratory Science 60th Annual Scientific Meeting.
21st - 25th August 06. Napier War Memorial Centre.

Report on the inaugural North Island Seminar - May 2006

The first South Island Seminar was held in 1964. Since then, rumours of the great success of this annual gathering have filtered through to those of us in the North Island, particularly over recent years as increasing numbers of intrepid northerners have ventured south to experience the southern hospitality at first hand. Last year there was some discussion around the Council table of the benefit of promoting a similar meeting in the North Island. In a rash moment, Tony Mace and I offered to organise the inaugural North Island Seminar, to be held Hamilton. A review of the Institute calendar suggested that a date in early May would be the most appropriate, and the 13th was selected.

In considering the aims and format of the meeting, we were keen to emulate the ethos of the South Island Seminar, which has a history of promoting stimulating professional interaction along with a memorable social event. As with the South Island Seminar, we also believed that it was important to encourage first and second time presenters, hopefully in an environment that was not too intimidating.

Coincidentally, the New Zealand Flow Cytometry Group was to hold its annual meeting in Hamilton. This was being ably organised by Barbara Harrison from the flow cytometry section of the Haematology Laboratory at Waikato Hospital. It was decided that there were some benefits to be had if the two meetings were held conjointly in the same venue. With this in mind, a number of conference venues in Hamilton were considered, and in the end the Kingsgate Hotel was selected. We had more than a passing acquaintance with this venue, and were familiar with the facilities, having run the 2004 Annual Scientific Meeting there in 2004. The conference rooms looked to suit our needs well, with a maximum capacity of 150 each, well within the number of registrants that we anticipated- little did we know!

One of the most gratifying aspects of the organisation of this meeting was the level of support from the diagnostics industry. Abbott Diagnostics offered a prize for the best overall presentation while Roche Diagnostics provided a prize for the best first time presenter. Unfortunately, as it transpired, there were insufficient first time presenters to offer this prize and Bronwyn and the team from Roche developed, as an alternative, a quiz based on the content of the scientific presentations. The prize for this was open to all registrants. Through the support of Radiometer, we were able to provide a bottle of wine in appreciation to each of the presenters. The evening's entertainment would not have been possible without the sponsorship of Dade Behring (band), Beckman Coulter (singers) and Roche Diagnostics (wine on the tables).

The manner in which the registrations were received was illuminating and certainly resulted in some anguish, when two weeks prior to the meeting only forty registrations had been received. The room to hold 150 suddenly seemed very large. Fran assured us that procrastination, or corporate lethargy (particularly from DHBs), inevitably produces a rush of last minute registrations. This was certainly confirmed when, within a week, registration numbers had reached 150. The Kingsgate agreed to accept 160 registrants as a maximum, but this was reached within a day and the figure was subsequently increased to 170. The room no longer had the aura of being overly capacious, and unfortunately some late registration applications missed out on securing a registration.

The call for proffered papers produced a programme with presentations covering a broad range of topics and disciplines. All present agreed that the papers presented were of a particularly high standard, and this was corroborated by those on the panel judging for the best overall presentation.

In the first presentation of the day Barbara Hoy presented a case of haemolytic uraemic syndrome (HUS). This case nicely demonstrated the importance of reviewing the laboratory results from all disciplines when attempting to elucidate the underlying cause of a microangiopathic haemolytic anaemia. Barbara reminded us that more than 90% of cases of HUS occur in children younger than one year, and that both TTP and DIC must be included in the differential diagnosis.

In a fascinating presentation David Shepherd demonstrated the use of speech recognition software in pathology. It was obvious that in recent years this technology has evolved considerably. The pathology specific dictionaries now available greatly improve the functionality and accuracy of speech transcription in the clinical laboratory. The particular product demonstrated is currently used in at least two pathology laboratories in New Zealand.

The case of a patient with *Sporothrix schenckii* was reviewed by Mary Hudson. It was noted that NZ native trees are subject to Sporothrix infection, which is introduced through pinhole borer damage. The patient presented was a "hangi maker" who had considerable contact with felled native timber. Infection with Sporothrix leads to the typical large ulcerated lesions on the arm. The growth patterns on Sabdex slope were described as 5-8mm dark brown downy thallus with buff coloured fringes and no diffusible pigment. Itraconazole is the agent of choice for the treatment of lymphocutaneous sporotrichosis, generally used until at least one month after the lesions have healed.

Rosemaree Redshaw presented an interesting case study of a woman presenting with an unexpected marked elevation of serum prolactin. Secreted by the anterior pituitary gland, the primary effect of prolactin is initiation and maintenance of lactation in the postpartum period. The patient described reported taking maxalon (metoclopramide), a dopamine D2 antagonist known to increase prolactin, 17 days prior to the prolactin measurement. It is usual for the prolactin level to fall to normal levels 2-4 days post maxalon. There was no record of any additional maxalon ingestion immediately prior to prolactin testing and the cause of the abnormal result was uncertain.

Elaine Booker deservedly won the prize for best presentation with her paper entitled "My dippy dippy heart: a case of *Corynebacterium* diptheriae endocarditis". Employing excellent graphics Elaine used the case of a ten-year-old Samoan boy to describe *C. diphtheriae* endocarditis, a condition characterised by large valvular vegetations, septic emboli and aneurysms. The point was made that the endocarditis

caused by *C. diphtheriae* is particularly nasty, may cause systemic complications and requires rapid diagnosis.

Pam Stewart described a patient presenting with abdominal distension, nausea and vomiting, subsequently shown to be attributable to lead poisoning. Recent ingestion of traditional Indian Ayurvedic medicines was the most likely cause of the markedly elevated serum lead levels. The presentation included a review of lead, focusing on historical, environmental and medicinal aspects. An analysis of the lead content of Ayurvedic remedies revealed levels as high as 13mg/dose.

In his presentation entitled "Bacillus mucosus capsulatus with a modern twist (Old bug, new tricks)", Murray Robinson gave a historical review of *Klebsiella pneumoniae*, an organism with a fine pedigree. He then went on to describe an increase in resistance to amoxicillin/clavulanate noted in recent *Klebsiella pneumoniae* isolates. The isolates were also resistant to trimethoprim. All appeared to be of a similar strain and predominantly localised to the population serviced by the laboratory. Murray commented on the implications, in particular the importance of fully identifying isolates and investigating changes in normal patterns of resistance.

"A tall story: acromegaly and gigantism" was the title for Margaret Matson's presentation which looked at the role of growth hormone and insulin-like growth factor and the disorders associated with either an over or under production of one of these growth factors. Growth factor assays, and treatment of the disorders were also included in the presentation.

A child presenting with an infected lesion on the knee, and symptoms consistent with tetanus was presented by Martine Carter. *Clostridium tetani* was not isolated from the sample, but 16S rRNA bacterial sequencing revealed *Clostridium celerecrescens*, an organism first isolated from a human sample in 2005. The point was made that routine phenotypic laboratory methods are often insufficient for correct identification of bacterial species that are uncommonly implicated in clinical manifestations.

An interesting case of acute myelomonocytic leukaemia secondary to etoposide for the treatment of previously diagnosed Ewing's sarcoma was presented by Peter Anderson. Cytogenetic and molecular studies demonstrated 11,q23 - t(9;11)(p22;q23) and an MLL gene rearrangement, characteristic of secondary AML. The WHO classification of AML was reviewed.

Standardisation of HbA1c in the Waikato region was discussed by Lyn Clarke. Poor correlation for HbA1c testing between POCT instruments and the reference laboratory initiated an evaluation of appropriate instrumentation for HbA1c testing, particularly for the regional hospital laboratories. The presentation described the introduction of the Primus PDQ. Implementation of the new instrument was associated with improvements in both the precision of the test and QC performance. Key benefits of the PDQ included simplicity of use, a reduction in sample size, excellent reagent stability, improved turnaround times and amenability to connection to an information system.

Danyang Li's paper was entitled "Alzheimer's disease (AD) and apolipoprotein E genotyping (Apo E)". In the presentation it was reported that, although approximately 50 genes have been reported to be associated with late onset AD, Apo E is the only one well-established genetic risk factor. Apo E is a polymorphic 299-amino acid protein that participates in lipid metabolism in the periphery and the central nervous system. The Apo E gene in humans contains three common polymorphisms, with the E4 allele considered a susceptibility gene form AD. The balance of the presentation described experience with an Apo E genotyping kit on the Roche Light-cycler.

In her presentation "The role of respiratory pathogens in otitis media", Nisha Singh described a study undertaken to determine the likelihood of a causative role for respiratory viruses in otitis media with effusion (OME), and whether there may be a seasonal variation of these viruses. The study also sought to investigate and compare traditional culture methods and molecular approaches in detecting the major otitis pathogens isolated from the middle ear effusions of children. Results of the study showed that otitis media infections are mostly polymicrobial and that *A.otitidis* is the most frequently detected bacteria by PCR in middle ear effusions. The samples were found to be negative for the respiratory viruses tested.

In a joint presentation entitled "When things go wrong on holiday", Dr Brian Dwyer and Dr Richard Massey described an Asian woman with late presentation of HIV/AIDs. Initially presenting as acutely unwell, the findings in the patient were those of the AIDS defining illnesses pulmonary cryptococosis and tuberculosis. An additional finding was myeloid hyperplasia of the bone marrow with erythroid suppression. Both the clinical and pathological findings were reviewed. In a following presentation, a series of interesting TB case studies from the Waikato Hospital TB laboratory was presented by Kathy Coley.

Mary-Ann Janssen questioned whether the annual certification of biological safety cabinets might simply result in a blind sense of confidence. The presentation compared the annual cabinet certification with the newly introduced methodology of KI discus. The latter procedure provides assessment of the effect of the environment on the cabinet, ensuring operator safety. The presentation was illustrated with case studies involving cabinets failing the KI discus test at Waikato Hospital.

The scientific programme concluded with a presentation from Ross Hewett on drug testing in schools, Jillian Broadbent gave an update on the NZIMLS CPD programme, with attention to the results of the recent IANZ audit of participants, and Chris Byram delivered a graphic presentation of his experiences with disaster victim recovery following the Bosnian war and the Sri Lanka tsunami.

In the evening, an excellent dinner was put on by the Kingsgate Hotel, well in keeping with the quality of the catering provided throughout the day. The evening's entertainment commenced during dinner with delightful solo and duet performances from Lyrica. These included light opera, songs from the shows and well-known standards. Following a short break, Austin Powers introduced himself and his sidekick Felicity. Austin provided a superb show with appearances by Abba, Freddie Mercury, Michael Jackson, Madonna and a somewhat scantily clad Cher, interspersed with Austin's singing, dancing and witty repartee. All who stayed for the dinner thoroughly enjoyed themselves.

It was heartening that this inaugural North Island Seminar was so successful. During the dinner there was a pronouncement from the Tauranga contingent (remarkably before too much wine had been imbibed) that they would host next year's Seminar. We all look forward to meeting there in a year's time.

Robin Allen

Recent abstracts from articles published in the *Australian Journal of Medical Science*, the official publication of AIMS

Mina A. A new model to calculate uncertainty of measurement for quantitative assays in clinical pathology laboratories. *Austr J Med Sci* 2005; 26(4): 140-9.

Uncertainty of Measurement (UM) can assist in the interpretation and comparison of local results against international diagnostic protocols, and should facilitate a reduction in health care costs and also help protect laboratories against legal challenges. Determination of UM for quantitative testing in clinical pathology laboratories is also a requirement for National Association of Testing Authorities (NATA) accreditation. A practical and simple to use statistical model has been designed to make use of data readily available in a clinical laboratory to asses and establish UM for quantitative assays based on internal quality control data and external quality assurance scheme results. Unlike many current models, the suggested model is based on both random and systemic error. The model has also been compared and verified against quality specification based on Biological Variation (BV).

Chung WY, Gardiner DL, Trenholme KR. Plasmodium falciparum and red cell polymorphisms. *Austr J Med Sci* 2005; 26(4): 150-6.

For malaria parasites to survive in the mammalian host, the merozoite stage of *Plasmodium falciparum* must recognise and attach to the host red cell. It must then successfully enter the cell and replicate. Invasion is a highly complex, multistep process and it has been demonstrated that *P. falciparum* can utilise alternative pathways for invasion of red blood cells. Although many proteins play an important role in invasion, their functional characteristics remain unclear. Erythrocyte blood group antigens are polymorphic, inherited, carbohydrate or protein structures located on the extracellular surface of the red blood cell membrane. We review the current state of knowledge about receptor/ligand interactions and the relationships between red cell polymorphism and protection against malaria.

Draper ADK, Wall KC, Devereaux RJ. A case of meliodosis from regional Northern Territory. *Austr J Med Sci* 2005; 26(4): 157-8.

A 57-year-old male patient presented to Katherine Hospital's Emergency Department complaining of a persistent cough, fever, shortness of breath and lower left-sided chest pain. His condition had been treated empirically with roxithromycin and penicillin in the community setting but to no avail. A chest X-ray was taken and upon examination revealed pneumonia in the left lower lobe. A heavily blood -stained sputum sample was collected and blood specimens were drawn.

Electrolytes, renal function and liver function results were unremarkable, however the C-reactive protein was markedly elevated and a neutrophilia with a substantial left shift was also observed. Ceftriaxone was added to the antimicrobial regime but the patient continued to remain febrile with a decreased appetite and insomnia. The patient's clinical history was further investigated and his occupation as a gold miner was revealed. Subsequently, *Burkholderia psuedomallei* was suspected as the causative agent of his unresolving pneumonia and the patient was transferred to Royal Darwin Hospital for a CT Scan and further management. *B. psuedomallei* was later isolated from the patient's sputum and blood culture specimens. The patient's pneumonia eventually resolved and he was discharged after 24 days of hospitalisation.

Wuthiekanun V, Peacock SJ. Laboratory diagnosis of meliodosis. *Austr J Med Sci* 2006; 27(1): 4-10.

Meliodosis is a serious infection caused by the soil dwelling Gram-negative bacillus *Burkholderia pseudomalli*. This disease is most commonly reported in north-east Thailand and the top of northern Australia where it is considered endemic. The most frequent picture is a septicaemic illness often in association with bacterial dissemination to distant sites, but possible manifestations are extremely broad ranging. Isolation of *B. psuedomalli* represents the diagnostic "gold standard". Gram stain and microscopy of clinical specimens has poor sensitivity, and the bacterial appearance is not specific. Culture is straightforward, although sensitivity can be increased by the use of selective media for sterile site samples. Serological tests have poor diagnostic accuracy in meliodosis-endemic areas where seropositity is common in the healthy population, but are more useful in the non-endemic setting. Use of molecular techniques to identify the presence of *B. pseudomalli* in clinical specimens has been described, but it is not routine in most diagnostic microbiology laboratories.

Chotivanich K, Silamut K, Day NPJ. Laboratory diagnosis of malaria infection - a short review of methods. *Austr J Med Sci* 2006; 27(1): 11-5.

Malaria is one of the most important tropical infectious diseases. The incidence of malaria worldwide is estimated to be 300-500 million clinical cases each year with a mortality of between one and three million people worldwide annually. The accurate and timely diagnosis of malaria infection is essential if severe complications and mortality are to be reduced by early specific antimalarial treatment. This review details the methods for laboratory diagnosis of malaria infection.

Smythe LD. Leptospirosis - a review of laboratory diagnostic methods. *Austr J Med Sci* 2006; 27(1): 16-9.

The diagnosis of leptospirosis remains largely in the realm of serology but methods such as the polymerase chain reaction (PCR) are quickly becoming more attainable as routine tools in the diagnostic and research laboratory. The selection of a diagnostic method is still influenced by the primary or tertiary level role of a facility. Culture of the organism for the clinical diagnosis has virtually disappeared but remains critical for epidemiological investigations where the disease is emerging or isolates are needed for nucleic acid based profiling. The microscopic agglutination test (MAT) remains the gold standard for serology but inconsistencies in the method exist worldwide and the dependency on regionally relevant live cultures in the antigen panels

impedes its standardisation and broader use in laboratories.

Lunt RA. Rabies and Lyssavirus: an Australian diagnostic perspective. *Austr J Med Sci* 2006; 27(1): 20-5.

Rabies in humans and other susceptible animals is an encephalitic disease syndrome caused by the eponymous virus and closely related lyssaviruses. Rabies-related viruses in Australia have been identified as Australian bat lyssavirus (ABLV), found to date only in bats and following two fatal human cases. ABLV circulates as two variants, isolated respectively from flying foxes and an insectivorous bat species (Saccolaimus flaviventris), both assigned to genotype 7. In addition, two human rabies cases have been attributed to genotype 1 rabies virus, following infection acquired overseas which manifested in patients on return to Australia. Rabies is a vaccine preventable disease. Awareness is the key and laboratory diagnostic procedures are a critical component. In this paper, the background to laboratory investigations for lyssaviruses is reviewed from an Australian context.

Blacksell SD. Serological and clinical diagnosis of dengue virus infection - a review of current knowledge. *Austr J Med Sci* 2006; 27(1): 26-33.

Dengue fever, dengue haemorrhagic fever and dengue shock syndrome are tropical diseases that cause significant disease burden. It is estimated that more than 2.5 billion people are at risk of infection and more than 100 countries have endemic dengue virus transmission. This paper reviews the available serological assays for the diagnosis of acute dengue virus infection, differentiation of primary and later infections, and their appropriate application depending on the setting. Dengue clinical syndromes and diagnostic criteria are also described.

Burford B, Blacksell SD. Diagnosis and management of tropical infections in travellers and expatriates at the Australian Embassy Clinic, Laos: experience in a limited-resource environment. *Austr J Med Sci* 2006; 27(1): 34-8.

The Lao People's Democratic Republic (Lao PDR) is located in South East Asia bordered by Thailand, Vietnam, Cambodia, Myanmar and China. There is a large resident expatriate community as well as many foreign travellers. Health care resources available to this population are limited to a few embassy-based clinics. This review presents a perspective on managing tropical infections in the context of an expatriate clinic in the Lao PDR. Patients present with a variety of tropical infections and are often treated presumptively with minimal local laboratory support. Other laboratory tests that may be helpful to make an accurate diagnosis are detailed and discussed.

Graves S, Stenos J, Unsworth N, Nguyen C. Laboratory diagnosis of rickettsial infection. *Austr J Med Sci* 2006; 27(1): 39-44.

Rickettsial diseases (including Q-fever) are difficult to diagnose both clinically and in the laboratory.

Serology is the main diagnostic modality for rickettsial diseases and micro immunofluorescence is the recommended serological method. However serology can be unreliable (negative) early in the illness when the patient presents for medical attention. This is when the first serum sample is usually taken. Serology on later sera (which may by now have become positive) is often not performed and thus the diagnosis is not confirmed by the laboratory.

Recently nucleic acid amplification (NAA) has been used in some (reference) laboratories and provides a diagnostic result during the patient's acute illness, before antibodies have developed. DNA detection by real time polymerase chain reaction (PCR) is extremely sensitive and specific. However, such tests are not widely available.

Tissue culture of rickettsiae is not a useful method of diagnosis because it takes to long, and is to risky for routine diagnostic laboratories. Direct staining for rickettsia is rarely used.

Answers to HSIG Journal questionnaire:

1. True
2. True
3. True
4. True
5. True
6. True
7. 100 lymphocytes
8. a) Enzyme analysis
   b) Electron microscope examination of a buffy coat blood sample
9. Juvenile subtype of Batten's disease (NCL3)
10. a) Enzyme analysis of white blood cells
    b) Fibroblast culture

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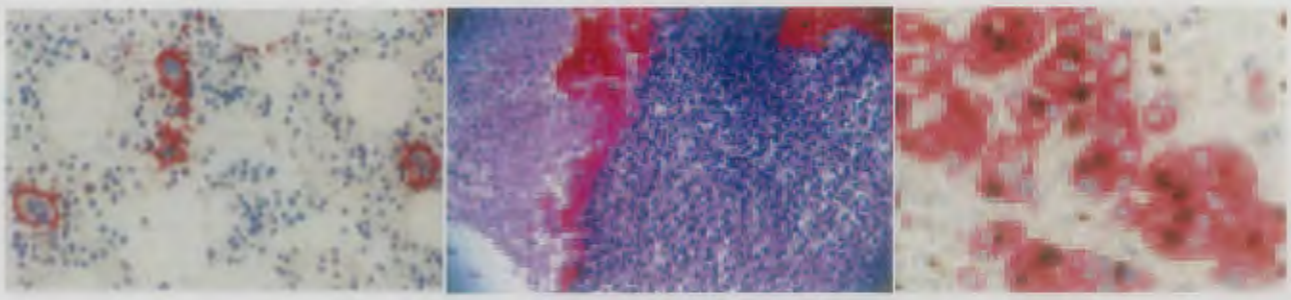

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