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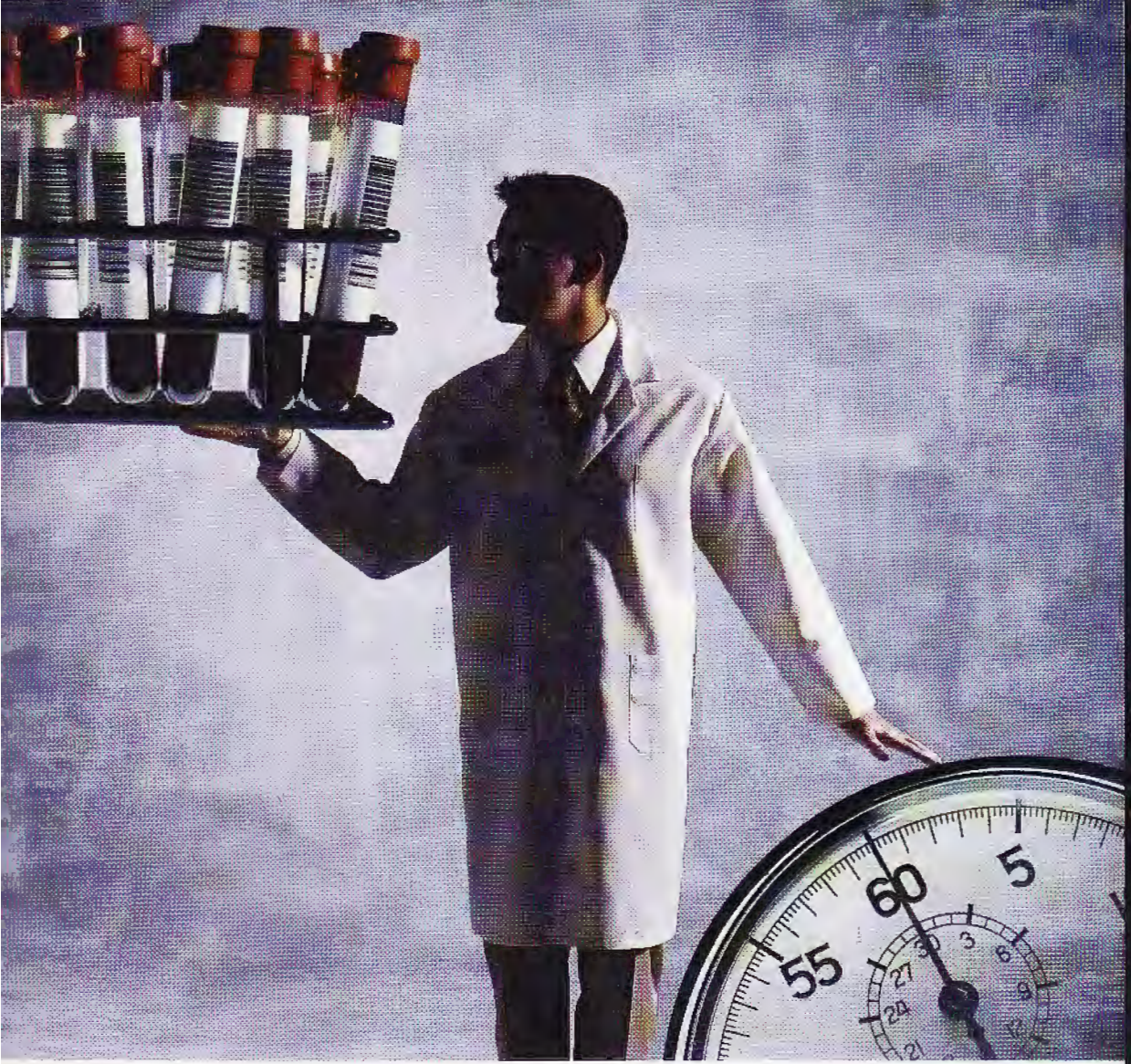

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* **Acknowledgements** should be made to people and/or organisations who have made substantial contributions to the study. Authors are responsible for obtaining consent from those acknowledged. Financial contributions towards the study from granting bodies or commercial organisations must be stated.

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New Zealand Development Assistance to the Health Laboratory Service in Binh Dinh Province, Vietnam.

Ron Mackenzie QSO, PhD, FNZIMLS
Pacific Paramedical Training Centre, Wellington

NZ J Med Lab Science 1999, 53(3) 2-?

The contribution of New Zealand in the development of the health laboratory and blood transfusion services in the Binh Dinh Province of Vietnam is unknown to many Institute members. It is an ongoing story which began in the early days of the Vietnam war as part of New Zealand's Colombo Plan aid to South East Asia.

Beginning in 1963, medical laboratory technologists were part of the New Zealand civilian surgical team working in the Provincial Hospital at Qui Nhou. The role of the technologists during these years was to provide basic laboratory tests and a blood bank facility for the surgical teams. With the expanding role of the teams in the late 1960s and improvement in the facilities, the laboratory became involved in additional work areas. These included public health investigations, blood transfusion service development and the establishment of a laboratory at the Holy Family Hospital which was later to become the Qui Nhou City Hospital.

Some nine New Zealand technologists served with the surgical teams over a period of 12 years until the war ended in 1975. To those involved during these years there are dark memories of a country at war but also many happy recollections of the Provincial Hospital, of friendships made and knowledge shared at the laboratory bench.

After the end of the war in 1975, the isolation of Vietnam internationally ended the good working relationship the New Zealanders had enjoyed in Qui Nhou and there was little opportunity for the laboratory to improve for more than a decade.

Towards the end of the 1980s the cold war thawed, international tensions were reduced and by 1993 Vietnam's isolation was largely over. Soon after this, diplomatic relationships were established between New Zealand and Vietnam and assistance to Vietnam became part of New Zealand's Overseas Development Aid Programme. It was at this time that a number of the former surgical team members, including medical laboratory scientists again looked towards Qui Nhou with a view to re-establishing contact and continuing the work which had been interrupted in 1975.

And so began the second part of the story in 1993 when the upgrading of the laboratory at the Provincial Hospital in Qui Nhou commenced. This project is now a well established part of New Zealand's aid programme in Vietnam. This also includes the development of the Bong Son Hospital laboratory and upgrading of the small district hospitals in the Bihn Dihn Province which together serve a population of close to two million people.

A major factor in the success in the Bihn Dihn laboratory assistance programme has been the New Zealand Vietnam Health Trust. The Trust was established in 1997 by the NZ Ministry of Foreign Affairs and Trade. This was achieved in large part by the initiative of former surgical team members who wished to see more coordination in NZ aid projects designed to improve the delivery of healthcare to the people of Vietnam in general but to those of the Bihn Dihn Province in particular.

To this end and in accordance with official aid policy the Trust was set up and given responsibility for the selection of appropriate medical aid projects and a budget for implementation.

The Trustees share a wide range of medical and technical expertise and to date have selected and overseen projects within the hospital, tertiary education and health science sectors. New diagnostic tools and techniques have been introduced, particularly in the laboratory, gastrointestinal, urological, thoracic, orthopaedic and ophthalmic surgery, and in the maternal and child health field.

The relevant skill and knowledge transfers have been affected in the main by specialists from New Zealand making short term visits.

Among the laboratory projects over the past three years have been inservice training and seminars at Bihn Dihn Province Hospital for Laboratory Technologists and provision of laboratory equipment. The inservice training has included workshops on clinical parasitology, microbiology and basic blood bank technology. A microscope maintenance programme has also been run and a supply arrangement for replacement parts established. A support programme for virus laboratories undertaking respiratory virus detection in Vietnam was undertaken and training for Vietnamese virologists provided in New Zealand.

The Trust has assisted with the long term VSA/LABNZ/PPTC initiative to upgrade the laboratory and blood transfusion service in the province and district hospitals with short term consultancy visits.

Two Province hospital Laboratory department heads have undertaken training courses in New Zealand and this year Gilbert Rose, a senior member of the Institute has completed an assignment in Qui Nhon in which he produced a complete set of bilingual laboratory manuals.

And so the story continues, what began some 36 years ago carries on still. Megan Smith has recently completed a two year VSA/LABNZ/PPTC assignment in the Laboratory at the Province Hospital in Qui Nhou and has been replaced by Elizabeth Denham who will carry on the laboratory development programme there, and in the district hospital laboratories in Bihn Dihn.

This is challenging work requiring both dedication as well as technical skill. The NZIMLS can well take pride in the achievements of the present generation of medical laboratory scientists who continue to work in Vietnam – Long may it continue.

Feasability of Pooling Faecal Specimens for Detecting the Presence of *Giardia* Antigen by Enzyme Immunoassy (EIA)

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Abstract
The feasability and performance, including sensitivity and specificity, of pooling faecal specimens for detection of *Giardia* antigen were evaluated using the ProSpecT *Giardia* Microplate Assay. The feasible number of specimens able to be pooled together was three. At this dilution the test showed acceptable sensitivity and specificity. When 300 specimens were pooled 3:1 into 100 wells, the assay had a sensitivity of 85% and a specificity of 98%. By increasing the number of true positive specimens to 80, the sensitivity of pooling was re-evaluated and showed an increase to 99%. Pooling faecal specimens is a sensitive and economical procedure for the detection of *Giardia* antigen using the ProSpecT *Giardia* Microplate Assay.

Keywords
Giardia, Pooling Specimens, Enzyme Immunoassay (EIA), Cost-Effective Testing.

Introduction
Giardiasis is one of the most commonly reported intestinal protozoan infections in humans. Outbreaks may occur as a result of drinking water contaminated by infected human or animal faeces. Traditionally, giardiasis has been diagnosed by microscopic detection of cysts and trophozoites in fresh or preserved faecal samples directly or with staining, with or without concentration.[1] *Giardia lamblia* may however be difficult to detect and in some studies only 50 to 70% of cases are identified by microscopic examination of one faecal sample[2]. The low sensitivity is mainly due to variable excretion of *G. lamblia.*

Immunoassay reagent kits are an alternative method to the routine "ova and parasite (O&P) examination" and provide added sensitivity which may allow the detection of infection in patients with low parasite numbers. Several recent studies have shown enzyme immunoassays (EIA) to be sensitive and cost-effective ways to detect *Giardia lamblia* in faecal specimens[3-5].

The ProSpecT *Giardia* Microplate Assay Alexon-Trend, Inc., Ramsey, USA, is a solid phase immunoassay using a monoclonal antibody for qualitative detection of *Giardia* Specific Antigen (GSA65) in aqueous extracts of faecal specimens. Anti GSA 65 antibodies have not been found to cross react with other enteric parasites. The ProSpecT *Giardia* Microplate Assay package insert claims sensitivities of 98% and 100% in trials of 248 and 562 specimens respectively[6]. Other studies have claimed sensitivities from 94% to 100% with specificities from 98.1% to 100% for the ProSpecT *Giardia* Microplate Assay[1,5].

Given the pressure laboratories are under to contain costs, we have examined the feasibility of pooling faecal specimens for the detection of *Giardia* antigen using ProSpecT *Giardia* Microplate Assay by: diluting two positive specimens and the positive control supplied by the manufacturer to determine the appropriate number of specimens which could be pooled; pooling 300 previously tested random specimens to determine the sensitivity and specificity of this pooling ratio; and diluting 80 selected positive specimens 1:3 to confirm the sensitivity of the assay.

Materials and Methods
Specimens
This study was performed on the faecal specimens submitted to Microbiology Department, Diagnostic Laboratory, for *Giardia* testing. Two positive specimens (one weakly positive and one stongly positive) by the routine EIA test were selected for the dilution with negative specimens. Three hundred random previously tested faecal specimens, 13 positives and 287 negatives, were retested when pooled 3:1. These specimens were stored at 2-8° C for up to 48 hours. Eighty previously tested EIA positive specimens were stored at -20° C for up to 1 month before retesting at a 1:3 dilution.

Methods
Testing was performed using the ProSpecT *Giardia* Microplate Assay (Alexon-Trend). All specimens were pre-diluted with Specimen Dilution Buffer (SDB) in tubes before adding to wells. Each specimen was prepared and tested according to the manufacturer's protocol apart from the modification to the number of specimens put into each well for the purpose of these experiments.

Dilution Trial. Two positive specimens and the kit's positive control were tested in a five step dilution series from a 1:1 to 1:5 using SDB.

Pooling Trial One. Three hundred random specimens were brought to room temperature before diluting with SDB. Three randomly chosen specimens were added into one well, 66µl from each specimen until 100 wells were filled with the 300 specimens.

Pooling Trial Two. Eighty frozen positive faecal specimens were defrosted at room temperature overnight before diluting with SDB. Each well contained 66µl from a known positive specimen and two negative specimens, 66µl from each, so that the positive specimens was diluted 1:3 for testing.

A true positive was defined as a positive optical density in the routine unpooled EIA method.

Results
Dilution Trial. Pooling an equivalent of up to 5 specimens together in one well gave a positive EIA results by the assay, Table 1. In the Auckland Diagnostic Laboratory testing region, the positivity rate for *Giardia* is approximately 4%. Using this figure, the average (expected) number of wells needed for each dilution for *Giardia* EIA (including the confirmation step using the routine unpooled method) was calculated, Table 1. The average number of wells needed for *Giardia* EIA reduced with the increased number of specimens pooled into one well. When any pooled well result is positive the individual specimen must be retested separately to confirm which specimen/specimens is/are the true positive/positives. The feasible number of specimens able to be pooled into one well for Giardia EIA was determined to be three. Therefore a dilution of 1:3 was used for the two subsequent pooling experiments.

Table 1. Effect of diluting positive specimens and the EIA kit positive control*

Dilution	High positive specimen	Low positive specimen	EIA kit positive control	Average (expected) number of wells needed for each dilution. $n/d + (1-(1-r)^d)n$+
1:1	>3(+)	0.93(+)	2.19(+)	n
1:2	>3(+)	0.805(+)	1.562(+)	0.58 n
1:3	>3(+)	0.329(+)	1.355(+)	0.45 n
1:4	>3(+)	0.185(+)	1.063(+)	0.40 n
1:5	>3 (+)	0.128(+)	0.731(+)	0.38 n

* Test results are expressed in Optical Density (O.D.) and interpretive result (+=Positive). The cut-off O.D. For a positive test was 0.05.

\+ n=number of specimens tested; d= dilution ration; r=positive rate (4%). Average (expected) number of wells for each dilution were calculated to 2 decimal points.

Pooling Trial One. Pooling three specimens into a single well had an effect on the sensitivity of the assay, Table 2.

Pooling Trial Two. When 80 previously tested positive specimens were retested diluted 1:3. one became a false negative result, Table 3. One specimen previously reported as positive appears to be a true negative specimen after confirmation tests using routine unpooled EIA method, Table 3.

By combining the results from the two pooling trials, the sensitivity of using pooled specimens for *Giardia* antigen detection (new test) was found to be 97%, 89/92, for the total of 380 faecal specimens tested. Only 3 true positive specimens were undetected when specimens were pooled for testing. The specificity remains unchanged at 100%.

Discussion

We determined that the feasible number of faecal specimens able to be pooled for *Giardia* antigen detection was three.This was chosen because it provides a safe margin for the assay to still detect the presence of *Giardia* antigen, given that even when diluted 1:4 and 1:5 the assay result was still positive, Table 1.

The sensitivity of *Giardia* antigen detection using 3:1 specimens (new test) was only 85% in the trial of 300 random specimens. This is less than the sensitivity quoted by the manufacturer, 98% and 100%, in their trials of 248 and 562 specimens by the routine unpooled method (reference test). The lower sensitivity by pooling specimens in this part of our study is a reflection of the small number, 13, of true positive specimens included. Failure to detect 1 or 2 true positives has a significant impact on sensitivity. Therefore, increasing the number of true positive specimens to ensure the increased sensitivity of the trail was proposed. In the second pooling trial, where 80 positives were diluted, there was a minimum decrease in sensitivity, Table 3.

The overall performance of the pooling trials for *Giardia* antigen detection was demonstrated by combining the results from the two pooling trials. The sensitivity for the new test using pooled specimens was 97% for the 380 specimens tested.

By pooling specimens, there is a significant reduction in the number of wells required for the assay. By using three specimens pooled into one well for testing, approximately half (46%) number of wells are used and, therefore half the amount of reagents are saved, Table 1. The reductions in number of wells and reagents used correlate proportionally to the lower cost of pooling specimens because the major cost in *Giardia* EIA testing is the reagent cost.

This study concludes that the pooling faecal specimens 3:1 is a sensitive and cost-effective approach to detect the presence of the *Giardia* antigen with the ProSpecT assay. Such an approach would reduce reagent costs by approximately 50%. While we have shown it is feasible to combine specimens for testing this goes against established protocols which endeavour to maintain integrity. The actual savings able to be achieved will depend on kit cost and volume of use because fewer kits are ordered this may offset any cost saving. Nevertheless given the difficulties laboratories have experienced in achieving increases in the price paid for tests there will be ever increasing pressure for cost containment. Pooling specimens is one way in which cost reduction may have to be achieved.

Table 2. The performance of the EIA assay to detect *Giardia* antigen in 300 specimens pooled in to 100 tests.*

		Routine EIA test result (Reference Test) +	-	
EIA result when	+	11	0	11
pooling specimens 3:1	-	2	287	289
(New Test)		13	287	300

* Sensitivity = 85%, specificity = 100%

Table 3. The performance of the EIA test on 80 *Giardia* positive specimens diluted 1:3.*

		Routine EIA test result (Reference Test) +	-	
EIA result after	+	78	0	78
pooling specimens 1:3	-	1	1+	2
(New Test)		79	1+	80

* Sensitivity = 99%, specificity = 100%

\+ One stool specimen previously reported positive was later confirmed to be a true negative specimen using the routine (unpooled) EIA method.

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Grateful thanks to the staff of Microbiology Department, Diagnostic Laboratory who assisted with these experiments, especially Michael McCarthy for his technical assistance and professional comments on this report. Grateful thanks to Elizabeth Robinson of Biostatistics Unit, Department of Community Health; who assisted with the statistical interpretation of this study.

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Proliferation Markers in Histopathology

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NZ J Med Lab Science 1999, 53(3):00-00

Abstract

The role of the pathologist is principally one of diagnosis; however the advent of molecular biology, which permits examination of tissue at a sub-cellular level, has provided pathologists with the opportunity of deriving information regarding disease outcome and progression. The most significant advances in this area have occurred in the development of techniques that allow the quantitation of cell proliferation in fixed tissue.

Some idea as to the proliferative activity of tumours may be obtained from assessment of tumour grade and mitotic rate, although these techniques are crude, objective and subject to artefactual variation. The identification of nucleolar proteins and various antigens associated with specific components of the cell cycle has facilitated investigations into tumour proliferation as a prognostic marker.

The proliferative rate of tumours is a function of two variables; cell cycle duration and the proportion of cells within a given tumour population that are undergoing cell cycle activity.

The duration of the cell cycle within a specific tumour may be measured by staining nucleolar organiser regions using a silver-colloid technique with gold toning. There are a variety of immunohistochemical stains that label cells within the proliferative component of a tumour cell population and, of this latter group of immunohistochemical stains, Ki-67 is the most specific cell cycle label.

Quantitation of nucleolar organiser regions or Ki-67 labelling will not in isolation provide an accurate assessment of cell cycle activity, as the parameters that these markers measure are independent of each other. Cellular proliferation studies therefore must include assessment of both nucleolar organiser region score and Ki-67 index, with the proliferative activity index of a tissue being the mathematical product of these two markers.

Key Words

Tumour, Malignant, Proliferation, AgNORs, Ki-67, PCNA, Cell Cycle, Prognosis

While the practice of medicine dates back to antiquity, the role of the pathologist in the management of disease is a relatively new one dating back little more than 100 years. Rudolf Virchow, the founding father of pathology, struggled to convince the medical establishment that the histological examination of tissue would aid diagnosis and in turn provide useful predictive information regarding disease progression and outcome. Virchow recognised that diseased tissues exhibited architectural and cytologic abnormalities and taken in combination, this derangement of both building blocks and structure was a diagnostic sign of inflammation or malignancy.

While the importance of histopathology as the prominent diagnostic modality seems obvious to us today, such has not always been the case as Virchow had many sceptics.[1] Pathology was predominantly a German science and outside Europe clinicians were slow to grasp the value of examining tissues under the microscope. Gradually histologic examination of tissues began to be accepted as being an indispensable component of the diagnostic process and by the turn of the century the role of the pathologist was firmly established.

Tumour Grade

A major step in the prediction of outcome in malignancy resulted from the important observations of Broders who noted that, while some tumours showed an accurate recapitulation of the tissue of origin, others appeared less well differentiated. Broders further noted that in some cases tumours were so poorly differentiated, the tissue of origin was no longer discernible.[2] Using squamous cell carcinoma of the lip as a model, he derived the first grading system for a malignant tumour, basing the grade on the proportion of well differentiated to poorly differentiated tumour cells within histological sections. He further speculated that poorly differentiated tumours were more rapidly proliferating than well differentiated variants, and because of this concluded that poorly differentiated tumours had a less favourable prognosis.

The idea that tumour outcome could be predicted by histological grading was enthusiastically embraced by pathologists and numerous grading systems have been proposed for a wide variety of tumours. There has, however, been little consistency in the definition of criteria employed in the grading of various tumours and while the majority of grading systems are based on nuclear pleomorphism, a wide variety of architectural and cytological features have been employed in the grading process.[3]

Broders' grading system introduced the concept that tumours show differing degrees of differentiation and that poorly differentiated tumours are more aggressive and have a worse prognosis. The proposed grading system did not take into account the fact that some tumours show focal de-differentiation and thus have compartments that exhibit varying rates of proliferation. This variation in grading throughout individual tumours is commonly seen and serves to introduce a lack of reproducibility into the grading process due to sampling variation.[4] In addition to this problem, grading of tumours is hampered by a lack of consistency in grading criteria – with many parameters included in composite grading systems having no predictive power on univariate analysis, and further by the introduction of interobserver error based on the assessment of subjective grading criteria.[3] While tumour grades are classified into groups according to defined criteria, in actual practice there is a continuum of grades within a series of tumours. Because division of tumours into grading categories is artificial, the process may either overestimate or underestimate the proliferative activity of a tumour, resulting in the upgrading or downgrading of tumours whose features naturally place them between grading categories. Finally, the utility of grading as a marker of cellular proliferation and tumour prognosis is diminished in many tumour series as the distribution throughout each of the grades is not linear but Gaussian and therefore most tumours fall into the middle grading category, where behaviour is least predictable. While grading does permit prognostic assessment for some varieties of malignancy, the results are not entirely satisfactory and in many cases grading does not provide independent prognostic information.

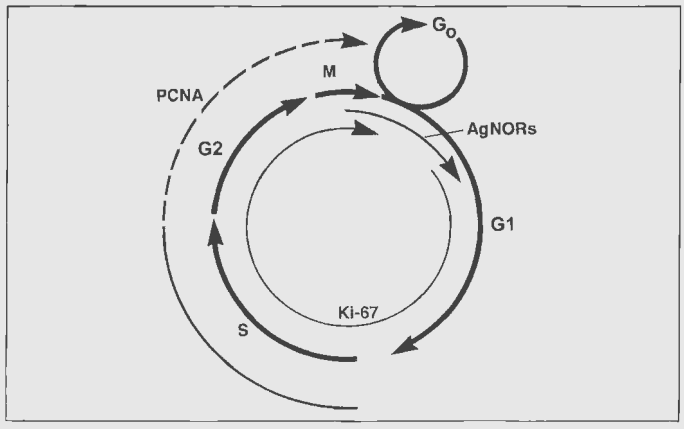

Figure 1. Phases of the cell cycle: G_0 – Resting phase, G_1 – Gap 1, S – Synthesis phase, G_2 – Gap 2, M – Mitosis phase.
AgNOR-phase of maximum dissociation of silver staining nucleolar organiser regions, Ki-67 –Ki-67 antigen expression, PCNA – Proliferating cell nuclear antigen expression.

Mitotic Rate

The quantitation of mitotic figures to produce a mitotic rate has long been considered a valid assessment of tumour proliferation and for some tumour types, such as smooth muscle tumours of the uterus and gastrointestinal tract, malignant behaviour is defined on the basis of mitotic rate.[5] Unlike tumour grade, mitotic rate directly assesses cell proliferation and has the advantage of being a continuous variable that is not reliant on arbitrary cutpoints. Analysis of mitotic index against patient survival, for a variety of tumour types, shows the parameter to have a significant correlation with survival, independent of nuclear grade and other prognostic features. In some tumours, such as malignant melanoma and breast carcinoma, assessment of mitotic rate can be used to assist in defining a composite tumour grade.

In practice, the application of mitotic rate as a prognostic parameter is hampered by several factors. Most importantly, mitoses occur over a relatively short period of the cell cycle and, may therefore, be infrequently encountered in slowly proliferating tumours, leading to the possibility of sampling bias.

While it is plausible to consider mitotic rate to be a marker of cell proliferation, the reproducibility of data derived form mitotic counts has been questioned. It is usual for mitotic figures to be assessed by light microscopy under high power (x400) magnification with rates being expressed as mitotic figures per high power field. This implies that all microscopes have the same field area, which is not the case[6] and further assumes that all tumours have the same degree of cellularity, with similar numbers of cells per unit volume or cross-sectional area. Various mathematical models and counting procedures have been derived to account for variations in tumour cellularity in the assessment of mitotic rate,[7] however these have not achieved widespread acceptance. In particular these models are not popular due to the necessary complexity of the mathematical formulations, which do not take into account heterogeneity of tumour morphology and the presence of compartments showing differing rates of proliferative activity.

Accurate quantitation of mitotic rate is further hampered by a poorly controllable variable associated with delay in fixation of the surgical specimen, as this results in decreased numbers of observable mitotic figures.[8] This phenomenon may be the result of evolution of initiated mitoses to the completion of that phase of the cell cycle or may be due to reduced identifiability of mitotic figures which resemble pyknotic nuclei if fixation is delayed.

The Cell Cycle

The observation that mitotic rate assessment is of prognostic significance for a variety of malignancies raises the possibility that direct markers of cell cycle activity may be of predictive value in the assessment of tumour outcome.

Tumour growth is not linear and not all cells show active proliferation. In any tissue only a small proportion of cells will be proliferating, while the majority will exist in a resting or non-proliferative state. In addition a small proportion of cells will exit either the cycling or resting population by apoptosis.

The cell cycle (Figure 1) is a complex and co-ordinated series of biochemical events that permits cell division to occur.[9] Quiescent non cycling cells (G_0) enter the cell cycle by activation of growth factors which bind to cell surface receptors. This part of the cycle is known as gap one or G_1 phase and is characterised by the activation of a signalling cascade that regulates the transcription of both immediate and delayed early response genes. In G_1 the cell is subjected to extrinsic growth factors, however, beyond the restriction point when the cell enters the synthesis (S) phase, the cell no longer responds to external controls. The S phase is characterised by replication of DNA and once this is completed the cell enters the gap two (G_2) phase where cytoplasmic proteins, organelles and RNA are synthesised. This prepares the cell for mitotic division (M phase) which lasts for 1-2 hours. Following completion of mitosis G_1 recommences, however, the cell can leave the cycle and enter the resting (G_0) phase any time up to commencement of DNA synthesis (S phase). The timing of a complete cell cycle varies from tissue to tissue. The duration of the S phase (7-12 hours), G_2 phase (1-6 hours) and the mitosis are relatively constant, however G_1 can show considerable variation and it is this phase which defines the length of the cycle.[10]

Proliferating versus Non-proliferating cells

Cell proliferation is associated with the generation of proteins which are necessary components of the biochemistry of cell cycle activity. The identification of these proteins by antigen-antibody labelling, using one of the accepted immunohistochemical labelling techniques, has provided the tool for identification of cells within the cell cycle.

Proliferating cell nuclear antigen (PCNA) is a polymerase ∂ auxiliary protein that is necessary for the elongation of primed DNA templates by DNA polymerase. PCNA is expressed in the late G_2 phase of the cell cycle with maximum levels being found on the S phase.[11] In early studies it was claimed that PCNA immunohistochemical labelling provided evidence of the S phase fraction of cells within a tumour. Unfortunately PCNA has not lived up to its early promise as a marker of cells within the cell cycle. The relatively long half-life of the protein means that expression can extend beyond the S phase into the M phase and beyond into G_0 phase cells in rapidly proliferating tumours.[12] The presence of PCNA protein within quiescent (G_0) cells militates against its utility as a cell cycle marker as this will result in an over-estimation of the cycling component of rapidly proliferating tumours.

The antigen Ki-67 has long been known to be a marker of cycling cells with the protein being expressed in all phases of the cell cycle (G_1 to M) but not in quiescent (G_0) cells.[13] Ki-67 antigen has been identified as a protein doublet of 345 and 395 kD. While the Ki-67 gene has been identified, the nature of the Ki-67 protein is unknown, although it has been speculated that it is a structural protein that maintains the higher order structure of DNA during mitosis.[14] Ki-67 protein has a very short half life and is expressed during, but not beyond, the cell cycle and is therefore an appropriate marker of proliferating cells. The specificity of Ki-67 as a cell cycle label has been recognised for some years, however, until recently the clinical impor

Figure 2. Individual silver staining nucleolar organiser regions (AgNORs) are visible within the nucleolus of a paratesticular adenomatoid tumour cell. Silver colloid stain with gold toning. Magnification x 1600.

tance of the antibody was hampered by its limited application in biopsy tissues. The earlier form of Ki-67 label was a monoclonal antibody that did not detect Ki-67 protein in fixed, paraffin-embedded tissue. This problem has been solved by the development of a polyclonal antibody that recognises an antigen that is not destroyed by routine processing of tissues. The development of a polyclonal antibody to Ki-67 protein has had the effect of permitting determination of the proportion of cycling cells within archival tissues and routine pathology specimens. To date the prognostic significance of Ki-67 expression has been successfully evaluated in a wide variety of malignancies and in some cases Ki-67 labelling has been used to distinguish between benign and malignant neoplasms.[15]

While Ki-67 is a marker of cell proliferation, it does not provide information as to the proliferation rate of cells that show positivity labelling, but merely identifies those cells that are beyond G_0 and in the pre-mitotic phase.

Cell Cycle Duration

Considerable interest was generated in the assessment of tumour proliferation as a prognostic marker by the development of silver-colloid staining technique for the staining of nucleolar organiser regions (AgNORs).[16] AgNORs are argyrophilic intranuclear non-histone proteins associated with loops of ribosomal DNA. In the nucleus, the early stages of protein synthesis occur in the dense fibrillary component of the nucleolus where there is elongation of the 45s-47s precursor RNA molecules. Nucleolin or C_{23} protein, a phosphoprotein necessary for the regulation of RNA polymerase I, is associated with the dense fibrillary component and it is this that constitutes AgNORs on silver staining.[17]

It has been shown that in the human karyotype, AgNORs are situated on the short arms of the acrocentric chromosomes 13,14, 15, 21 and 22. Interphase nuclei contain a maximum of 10 AgNORs and this increases to 20 during mitotic interphase in normal cells, however in rapidly proliferating neoplastic cells, many more AgNORs are seen.[18] In rapidly proliferating cells there is less time available for histone protein synthesis prior to mitosis and this is facilitated by activation of a greater number of ribosomal DNA sequences. Ribosomal DNA transcription requires AgNOR proteins in order for protein synthesis to occur and for this reason activation of ribosomal DNA is accompanied by increased amounts of AgNOR-protein.[19] The silver -colloid technique is of clinical value as assessment of AgNOR numbers correlates with cell cycle duration and thus provides an insight into a dynamic process in fixed tissue.

The development of the silver-colloid method and its use in paraffin-embedded tissue has led to intense investigation into possible clinical applications of this technique in both benign and malignant tissues.

The technique is however somewhat capricious and in a number of studies there has been considerable disagreement as to the observed numbers of AgNORs in similar series of tumours.[20] These problems have arisen due to the lack of an agreed definition as to what constitutes an AgNOR for counting purposes and have been compounded by poor staining techniques resulting in obliteration of small AgNOR clusters. The problem of overstaining has been overcome by the addition of a gold toning step to the silver-colloid process which has the effect of sharpening staining of small AgNORs and reducing background silver deposition.[21] There is also now general agreement that all argyrophilic foci within a nucleolus or nucleolar fragment (Figure 2), in addition to solitary AgNORs within the nucleus (satellite AgNORs) should be counted in order to provide an aggregate AgNOR score for each of the cells examined.[22]

Using these techniques various studies have shown enumeration of AgNORs to provide clinical information that is of diagnostic and prognostic importance.[23]

Derivation of a Proliferation Index

Quantitation of Ki-67 immunoexpression and AgNOR staining in tumours provides prognostic information that is both significant and additive, as the former is a marker of the cells in the tumour proliferation compartment, while the latter is a direct measure of cell cycle duration.[24] The two techniques are therefore complimentary and permit the derivation of a proliferation index that is the product of the two components;

Ki-67	x	AgNOR score	=	Proliferation rate
[% of cycling cells]		[duration of cycle]		

Although Ki-67 indices and AgNOR scores have been used in isolation to predict tumour behaviour, this composite prognostic index, which reflects tumour proliferation, is a novel concept that has been validated in few tumours. Further developments in this area are awaited, however early results are promising and suggest that the assessment of a proliferation index will provide prognostic data that is superior to both tumour grade and mitotic rate.

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The Inappropriate Use of Statistics

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Abstract
The proliferation of powerful statistical software for personal computers has seen a similar growth in the number of statistical techniques employed in the medical literature. Here we review common problems in the application of these techniques. Basic statistical terminology and techniques are briefly reviewed. Errors in the design and conduct of scientific trials can be grouped into four broad areas:
1. Experimental design and conduct of the trial;
2. Analysis of the data;
3. Interpretation of results, and
4. Presentation of the results.

Common problems in trial design and conduct are examined in terms of appropriate sample and control selection (including sample size), collection of data and retrospective study designs. Numerous common analytical errors are presented with more appropriate alterntives. Common errors in the interpretation of statistical results include confusion between clinical and statistical significance, incorrect application of *P* values, and the belief that statistically significant association provides direct evidence of a causal relationship between the variables concerned. Finally, common data presentation errors and some special problems of interest to clinical scientists are examined (reference intervals and method comparisons). We conclude that the increasing availability of powerful statistical methods is not matched by an equivalent level of understanding in their appropriate application. These increasingly prevalent errors can mislead other investigators, lead to inferior or delayed patient treatments, and perpetuate similar errors in the literature.

Keywords
Statistical errors, inappropriate statistics, reference interval, method comparison.

Introduction
A variety of statistical techniques are being used increasingly in the medical literature, reflecting both a preference by editors for quantitative data and a wider availability of statistical software packages. However, the application of these techniques has frequently been criticised as inappropriate, by a number of authors (1-3). For example, Tyson *et al.* (4) reviewed 86 therapeutic trials conducted in the field of perinatal medicine and found the statistical problems highlighted in Table 1. It should not be inferred from this data that researchers in the field of perinatal medicine are any less skilled than those in other branches of medicine, but rather that these are characteristic of the types of errors widespread in the medical literature.

This paper seeks to describe the inappropriate use of statistics in the medical literature. Statistical tests will not be described in detail. Readers are directed to other reviews and texts for this purpose (5-9). Fundamental statistical concepts will be described, where necessary, to provide a background for understanding particular errors. There are at least two approaches for demonstrating inappropriate statistical testing. One way would be to list, in dictionary form, various examples of incorrect usage as found in the literature. The second means is to systematically evaluate where, and why, these errors occur. The latter path has been chosen, although examples will be provided where this is possible.

One point should be stressed at the outset of this review. Rothman (10) wrote *"many medical researchers believe that it would be fruitless to submit for publication any paper that lacks statistical tests of significance"*. It is not always necessary to include a statistical analysis in every paper. A statistical test is used to test a hypothesis which has resulted in a controlled experiment being conducted. In many observational studies which are exploratory in nature no hypothesis is tested, but explanations are proffered for observed effects. It is inappropriate to employ statistical tests simply because the researcher believes no study is complete without them.

Hypothesis or significance tests
Two statistical concepts that must be understood before continuing are those of hypothesis testing and confidence intervals. In order to answer a specific question such as *"is this method better than our current procedure?"* it is often necessary to collect data and test a hypothesis. Usually, to introduce some caution before an effect is claimed, the hypothesis is that *there is no difference*. This is called the *null hypothesis* and the data are analysed using a *significance or hypothesis test* to support or refute this hypothesis. Data is then selected from the population of all possible points and tested to see if the values obtained would be 'unlikely' to arise, if the null hypothesis were true.

Table 1. Summary of review of 86 therapeutic trials in perinatal medicine (Tyson *et al.* 1983)

Statistical problem	% of studies fulfilling criteria		
	Yes	Unclear	No
Statement of purpose	94	6	0
Clearly defined outcome variables	74	1	25
Planned prospective data collection	48	30	22
Predetermined sample size (or a sequential trial)	3	16	71
Sample size specified	93	6	1
Disease/health status of subjects specified (n=85)	51	20	29
Exclusion criteria specified (n=81)	46	9	45
Randomisation (if feasible) appropriately performed and documented (n=69)	9	12	79
Binding used, or lack of binding unlikely to have biased results (n=83)	49	47	4
Adequate sample size	15	44	41
Statistical methods identified, appropriately used and interpreted	26	0	74
Recommendations/conclusions justified	10	71	19

The significance test is a rule for deciding whether any particular sample is in the 'unlikely' class, or more usefully, for assessing the strength of the conflict between what is found in the sample and what was predicted by the null hypothesis. The dividing line between the 'likely' and the unlikely' classes is usually defined in terms of a probability *P*, which is referred to as the *significance level.* A result would be called significant at the 5% level if the sample was in the class containing those samples most removed from the null hypothesis, when that class contains no more than 5% of all possible samples. *P* is the probability of obtaining a result at least as unlikely as the observed one, if the null hypothesis is true. It should be realised that the level of significance is simply an indication of the 'degree of plausibility' of the null hypothesis.

P values and confidence intervals

A common problem with many 'rusty' statisticians is confusion between *P* values and confidence intervals. As described above, a *P* value is the probability that the observed data have arisen purely by chance, when the null hypothesis is true. One false interpretation is that a *P* value of, say, 0.001 implies a stronger effect than *P*=0.01. the numerical difference in the *P* values does not support this conclusion. A statistical test assesses, by means of the probability *P*, the plausibility of the observed data, when some null hypothesis is true. If the *P* value is large, the data are consistent with the null hypothesis. *P* is not the probability of there being no real effect. Highly significant *P* values can accompany negligible differences (if the study is large), and unimpressive *P* values can accompany strong associations (if the study is small). A sole *P* value fails to convey adequately the findings of many trials.

A confidence interval can be thought of as the set of true but unknown differences that are statistically compatible with the observed differences (11). True differences that lie outside the confidence interval (usually set at 95%) are not impossible; they merely have less evidence supporting them than values within it.

Confidence intervals answer the question "how large is the effect" rather than the question "is there an effect" which is answered by the use of *P* values. There are two ways of interpreting the confidence interval:

1. The values of the parameter inside the 95% confidence interval are precisely those which would not be contradicted by a significance test at the 5% level, because there is only a probability of 5% that the interval does not contain the population value. Values outside the interval would all be contradicted by a two-sided test at the 5% level.
2. The confidence interval contains the population value with a probability of 0.95.

It follows from 1 that a confidence interval may be regarded as equivalent to performing a significance test for all values of a parameter, not just a single value corresponding to the null hypothesis. The addition of the concept of an 'important difference' to that of a null hypothesis means that there are four possible interpretations to an analysis (12):

a) the difference is significant and large enough to be of practical importance;
b) the difference is significant but too small to be of practical importance;
c) the difference is not significant but may be large enough to be important; and
d) the difference is not significant and also not large enough to be important.

The size of the difference which is large enough to be of practical importance will be based on commonsense and professional judgment. The problem with a significance test is that it may fail to detect a real effect; that is the null hypothesis is false but the evidence is not strong enough to reject it. This error is called a *Type II error.* A *Type I error* occurs when real effects are claimed when the null hypothesis is true. The usual symbolic representation for the probability of these errors occurring is β and α respectively as described in Table 2. Usually α is set to 5% in advance and is called the level of significance of the test. β is dependent on the sample size and size of the effect one is interested in. β can be set in advance by choosing an appropriate sample size and utilising the concept of power. A wide confidence interval is an indication of a low power. Optimal sample size and statistical power will be examined in greater detail below.

Significance tests can be either *one-sided* or *two-sided.* A two-sided test means that sufficiently large departures from the null hypothesis in either direction will be judged significant. A one-sided test at a level *P* is therefore the same as a two-sided test at level 2P, except that departures fromt he null hypothesis are only counted in one direction.

Table 2. Types of conclusions in statistical hypothesis testing

	Actual situation:	
Conclude from observations	**Treatment has an effect**	**Treatment has no effect**
Treatment has an effect	True positive, correct conclusion ($1-\beta$)	False positive, Type I error (α)
Treatment has no effect	False negative, Type II error (β)	True negative, Correct conclusion ($1-\alpha$)

Sources of inappropriate use

In the design and conduct of a scientific trial, the areas where error does occur can be grouped into the following three broad areas:

1. Experimental design and conduct of the trial;
2. Analysis and interpretation of the data; and
3. Presentation of the results.

There is an obvious link between these sources of error and the various steps in classical hypothesis testing. It is worth describing the generic research design (after Lewis and Bessen (13) and incorporating into these steps the categories of error listed above (see Table 3). We shall now investigate the sources of inappropriate statistical usage, beginning with the first category, errors in design and conduct of scientific trials.

Table 3. The basic steps of a scientific trial with the associated sources of appropriate use of statistics.

Errors in design and execution:

1. Determine the statistical form of the data to be collected; discrete or continuous (normally distributed or not?). Ensure outcomes are measured (responses) and study factors (exposures) are identified.
2. Define the null and the test hypothesis, the two possible conclusions to be distinguished.
3. Collect the data.

Errors in analysis and interpretation:

4. Calculate the probability of the results occurring if the null hypothesis were true, using a statistical test. The type of statistical test depends on the results of step 1.

5. If this probability is small (for example P< 0.05), reject the null hypothesis as false.

Errors in presentation:

6. Publish the results of the trial.

Errors in the design and execution of scientific trials

These errors are the most common and fundamental. A close reading of many published trials reveals sub-optimal experimental design (9). The problem with inferior research design is that outcomes are difficult to interpret and open to bias, particularly in that they may produce over-optimistic findings. A successful experimental design should provide both *internal* and *external validity. Internal validity* means that a cause and effect relationship can be elucidated and directly attributed to a specific treatment of the group. *External validity* describes the extent to which the findings of the study can be generalised to other populations or measurement variables. While the researcher should strive for strong internal and external validity, this is not always possible. Internal validity should always be sought as a minimum requirement. The reader is referred elsewhere for a more detailed examination of these research design issues (14, 15). We shall, however, briefly examine some common errors, to which all designs are susceptible.

1. Selection of a random sample: Statistical methods allow us to estimate the parameters of a population from a random sample of its members. Furthermore, we can also derive confidence ranges for these parameters. If the sample is not truly drawn at random from the population, then the logic underlying the distributions upon which the common test statistics (*F, t, X^2, r*) are based becomes invalid or at least only approximately true. The resulting *P* values are inaccurate – we are unable to determine if the observed differences were due to chance, or in fact as a result of the experimental treatments.
2. Appropriate sample: As well as the randomisation problem, there is often a question of what actual population the sample in any given study represents. Identifying the population in question is crucial in deciding the broader applicability of the study findings (i.e. external validity).
3. Appropriate control group: To reach meaningful conclusions about the efficacy of some treatment, one must compare the results obtained in the individuals who receive the treatment with an appropriate control group. Ideally, the *control group* should be identical to the treatment group in all respects, except the treatment. A statement of how the matching was achieved should always be present. Clinical studies often fail to include adequate controls. This omission generally biases the study in favour of the treatment.
4. Data collection – problems: There are a few problems that can arise when the trial data are being collected and analysed. The first of these is that of 'outliers', or more correctly, how to identify what is an outlier (16). Outliers can be defined as data values that are incompatible with the rest of the data. The great temptation with these points is to ignore them, but this will clearly affect the veracity of the analysis. The challenge is to differentiate a true extreme value from an erroneous value. Various outlier tests are available for both normally distributed and distribution free data sets (9, 17). These should be used, rather than arbitrarily eliminating some points. The other problem that may arise with data collection is that of missing values. In most cases a few points can be omitted without affecting the results. However, if there is a subject for whom there are some results missing and some collected, it is generally best to exclude all the data for this subject (16).
5. Retrospective studies: The foregoing discussion assumes that an experimental design is followed, however many studies are not actually designed, but rather 'happen'. They are based on the analysis of pre-existing data that were collected for some other purpose. This approach is called a *retrospective study,* because it involves looking back over previous experience to obtain data for analysis. Unfortunately, many reports of such studies do not admit that the study was retrospective or that the idea came before the data. Retrospective studies are prone to two potentially serious problems.

   First, the groups may vary in ways the investigators do not notice or chose to ignore. These differences, rather than the treatment itself, may account for the differences the investigators find. That is, the association between an exposure and an outcome is investigated but the exposure and outcome are strongly positively associated to a third variable. (These factors are called *confounding effects*). In such an observational study, nothing can definitely distinguish between these two interpretations.

   Secondly, such studies can be subject to bias in patient recall, investigator assessment, and selection of the treatment group and, often more important, the control group. These problems are especially difficult in studies based on chart reviews when the people reading the charts often must use considerable judgement in assessing what actually happened to the patient.

   Retrospective studies do have the advantages that they are relatively inexpensive. It is possible to accumulate enough cases to perform a meaningful analysis in a relatively short time, and, ethical considerations or prevailing medical practice can make it impossible to carry out active manipulations of the variable under study. Julious and Mullee (18) provide other examples.

   Some of the problems inherent in retrospective sutdies can be overcome if the investigators explicitly specify the criteria they used for classifying each subject in the control or treatment group. Such specifications help minimise biases when the study is done as well as help you, as a consumer of the resulting information, judge whether the classification rules made sense.
6. Sample size: All researchers planning trials are confronted with the question 'what is the appropriate sample size to choose?". Reviews by Freimann *et al.* (19), Goodman and Berlin (20), and Hall (21) have shown that many published clinical trials that found a non-significant difference between treatments had in fact little chance of detecting major treatment effects due to small sample sizes. According to Kingman (22), 'between 250,000 to a million trials of different treatments worldwide have already been completed, many of them too small to provide statistically significant results on their own".

   The *power* of a study is defined as the chance that the study will demonstrate a significant specified difference between the two groups when in reality there *is* a difference between them. For example if a study has a power of 80%, there is a 20% chance that the true difference between the groups will be missed. Few published studies report that the sample size was chosen on the basis of power calculations. Indeed, the concept of sample size calculations seems almost unkown in medical research outside the field of clinical trials, although the same methods are equally applicable to all comparative studies and can be used in planning any investigation. A description of the use of power in determining an adequate sample size is given by Sachs (17) and Bach and Sharpe (23).

Table 4. Summary of key statistical tests

Scale of measurement	Two treatment groups consisting of different individuals	Three or more treatment groups consisting of different individuals	Before and after a single treatment in the same individuals	Multiple treatments in the same individuals	Association between two variables
Interval (and drawn from normally distributed populations)	Unpaired *t* test	Analysis of variance	Paired *t* test	Repeated-measures analysis of variance	Linear regression and Pearson product-moment correlation
Nominal	Chi-square analysis of contingency table	Chi-square analysis of contingency table	McNemar's test	Cochrane Q	Contingency coefficient
Ordinal	Mann-Whitney rank-sum test	Kruskal-Wallis statistic	Wilcoxon signed-rank test	Friedman statistic	Spearman rank correlation

Even when power calculations have been used to calculate sample size, the supply of subjects may not be as great as anticipated. It is common in clinical trials for the actual recruitment rate to fall far short of that anticipated, partly because of overestimation of the number of eligible subjects and partly because of their unwillingness to enter the trial.

Errors in analysis

These errors occur when incorrect statistical tests are applied. To determine which test is appropriate, one needs to consider the experimental design. Were the treatments applied to the same or different individuals? How many treatments were there? Were all treatments applied to the same or different individuals? Was the experiment designed to define a tendency for two variables to increase or decrease together? How the response is measured is also important. Were the data measured on an interval scale? If so, are you satisfied that the underlying population is normally distributed? Do the variances within the treatment groups or about a regression line appear equal? When the observations do not appear to satisfy these requirements – or if you do not wish to assume that they do – you lose little power by using non-parametric methods based on ranks, although there appears a general reticence on the part of many scientists to use non-parametric statistical tests.

Finally, if the response is measured on a nominal scale in which the observations are simply categorised, one can analyse the results using contingency tables. Table 4 provides a summary of statistical tests and situations where they should be used (5).

It is worthy of mention that nominal or ordinal data will usually require non-parametric methods. What are nominal and ordinal data? Statisticians refer to three kinds of data: interval scale, nominal scale and ordinal scale. Quantitative numerical measurements of a property are examples of interval data. Qualitative attributes such as sex and occupation are nominal scale data. If data can be sorted into ordered categories (e.g. age categories) then it is said to be ordinal.

Altman (9) has summarised the common statistical errors occurring in the medical literature. We have used this as a framework to illustrate some specific errors in analysis and interpretation.

1. Using methods of analysis when the assumptions are not met: Failure to meet the requisite assumptions underlying statistical tests can lead to inaccurate and misleading findings. This common error stems from a poor understanding of the limitations of statistical tests. For common parametric tests (e.g. *t* test, linear regression), assumptions include the requirements that the populations from which the samples were drawn must be normally distributed, and the variances of each population must be equal even when the means are different. It may be possible to assume normality under the Central Limit Theorem where the sample size is sufficiently large. Other assumptions, applicable to most tests, are that samples be independent of each other, and that each sample be randomly selected from the population being studied.

The regression equation should not be used to extrapolate values beyond the original data range and linear regression should not be used where the data shows curvature. There are other specific requirements for particular procedures and these should be checked before a particular test is applied. Where a lesser known test has been applied, it should be described in some detail. Particular examples of these errors applicable to *t* and X^2 tests are described in Tables 5 and 6 (1-3, 24).

One common assumption is that a population is normally distributed, but this assumption is rarely validated. Data can be tested for significant deviations from the normal distribution by applying the Kolmorogov-Smirnov test (17) or Shapiro Wilk test (17).

Table 5. Commonly violated assumptions for the *t* test

Problem	Consequences
Lack of normality	The *t* test is reasonably robust for moderate departures from normality, but in small samples with skewed distributions it may be necessary to either transform the data or use a non-parametric test. Few authors consider either of these possibilities.
Equality of variances	For the use of the unpaired *t* test, the variances of the two samples should be equal although this is not essential if the samples are large and

	approximately the same size.
Independence of observations	Each sample must be independent of the others and hence if one sample has been matched with another, for example a control, the unpaired *t* test is inappropriate.
More than two groups	(See item 2 below, for X^2 test).

Table 6. Commonly violated assumptions for the X^2 test

Problem	Consequences
Null hypothesis	The X^2 test requires a null hypothesis to be stated or at least be able to be deducted from the context. Where this is not the case, the test has no meaning and any conclusions are unsupported.
Small numbers	In a 2 x 2 table with small numbers (e.g. the sample size is less than 20, or less than 40 with at least one expected value less than five), Yates, continuity correction or the Fisher Exact test are appropriate.
Independence of observations	If the groups are matched, the analysis should reflect this.
Use of continuous data or percentages	Only discrete data classes are appropriate for the X^2 test (Continuous data corrections available).
Testing of differences	In a paired study, mean differences should be tested against zero, rather than testing for differences between means.

2. Analysing paired data, ignoring the pairing: This situation can arise when the same individual (or matched individuals) have two different treatments, but the means of the two groups are compared rather than the individual differences. The use of paired tests is preferable wherever possible. These tests tend to have greater statistical power. Where the data are unpaired, the two sample *t* test should be used, whereas with paired data the paired *t* test is appropriate. Where non-parametric tests are to be used, the Mann-Whitney U test is appropriate for unpaired data, the Wilcoxon test is used for paired data, and the X^2 test for 2 x 2 tables is replaced by McNemar's test (25).
3. Failing to take account of ordered categories: Ordered categories involving data such as 'worse', 'unchanged' or 'improved' frequently occur in clinical medicine. these are often analysed by means of the X^2 test, however such an analysis may be insensitive to trends existing across the data. More appropriate would be either use of the Mann-Whitney test, or application of scores to the ordered categories together with the X^2 test for trend. Moses *et al.* (26) found in a review of articles published in The New England Journal of Medicine that although approximately 20% of research articles contained ordered category data, none of these had been analysed in the statistically preferred way.
4. Treating multiple observations on one subject as independent: the most common method of analysing multiple observations is to perform independent analyses at each time point using tests such as two-sample *t* tests, the Mann-Whitney test, or ANOVA. Frequently such data are displayed graphically by joining the mean values at each time point, typically with 'error bars' of ± 1 standard error (or perhaps ± 1 standard deviation). Altman (9) is highly ciritical of this approach, in that it fails to take advantage of study, variations between different individuals may be masked, it is very difficult to interpret the multiple *P* values obtained from comparing different subject groups, and finally no allowance can be made for missing observations.

   More appropriate techniques include repeated measures analysis, or the reduction of subject data to a fitted statistical model or summary data that can be analysed as if they were the original observations (27). Summary statistics themselves are however also open to problems, including:
   a) It may be difficult to specify the feature(s) of major importance, because the study objective is too vague;
   b) The choice of statistics to use may be influenced by inspecting the data;
   c) It is difficult to study any possible variation between groups in the shape of the curves (but this is always difficult).
5. Using multiple paired comparisons instead of an analysis that cosiders all groups: This effect is known as the multiple comparison problem, and can be seen in situations where a large number of significance tests are carried out on one set of data. The danger with this approach is that some statistically significant effects may occur purely by chance (28). For example, it may be more appropriate to use a one-way ANOVA rather than a series of *t* tests. Where multiple tests are performed be aware of 'data mining' – all planned analyses should be performed and reported, not just those that are significant.
6. Performing within group analyses and then comparing groups by comparing *P* values or confidence intervals: Randomised allocation in a trial does not guarantee the treatment groups are comparable with respect to baseline characteristics. It is vital that the correct experimental unit is identified for design and analysis. Whilst it is common to assess any differences between treatment groups with significance tests, these tests will actually only assess the randomisation (29).
7. Quoting confidence intervals that include impossible values: This practice raises questions regarding the credibility of the research. Such values may arise from blindly accepting the output from a statistical software package.
8. Using correlation in method comparison studies: Regression and correlation are separate techniques serving different purposes and need not necessarily accompany each other. Method comparison studies are frequently mis-analysed (30). There are a variety of techniques available, and studies comparing two (or more) methods are common. The aim of these studies is usually to see if the methods 'agree' well enough for one method to replace the other, or perhaps for the two methods to be used interchangeably. The same considerations apply to studies comparing two observers using one method. Note that we need to define what we mean by agreement. Also, we are concerned with the degree of agreement, so that this problem is one of estimation rather than hypothesis testing.

   In particular, the agreement between two methods is often interpreted as good if a high value of *r*, the correlation coeficient, is obtained (24). Correlation is an inappropriate analysis in this situation because the correlation coefficient is a measure of the strength of *linear association* between two variables, which is not the same as a measure of *agreement.* Agreement should be assessed in terms directly related to the

measurements. A high value of r can be obtained because there is large variation between subjects. It is clearly not reasonable to assess agreement by a statistical method that is highly sensitive to the choice of sample subjects. Another common incorrect analysis is the comparison of means by a hypothesis test, often a paired t test. We cannot deduce that methods agree well because they are not significantly different. Indeed a high scatter of differences may well lead to an important difference in means (bias) being non-significant. By this approach, worse agreement decreases the chance of finding a significant difference and so increases the chance that the methods will appear to agree!

If you assume that the aim of these types of investigation is to see if the two methods (or observers) *do not* agree, then it makes sense to analyse the differences between the measurements by the two methods on each subject. In this case, the mean of the differences will be a measure of accuracy and the standard deviation, a measure of imprecision. A fuller discussion of method comparison studies is given by Bland and Altman (30), and later in this review.

9. Using correlation to compare two sets of time-related observations: This is another misuse of correlation which can lead to spurious findings. The more appropriate analysis involves the use of summary statistics, as described previously (9).
10. Assessing the comparability of two or more groups by means of hypothesis tests: Hypothesis tests are used to evaluate preformed hypothesis. Other tests, suggested by a preliminary inspection of the data, will give a false impression because the calculated P value will be too small (31). It is not acceptable to test groups of results unless there is a reason for suspecting a difference; that is, the hypothesis should precede the statistical test, not the other way around.
11. Evaluating a diagnostic test solely by means of sensitivity and specificity: To evaluate a diagnostic test it is necessary to know the probability of the test giving an accurate and correct result, that is, the *positive* (and *negative*) *predictive value* of the test. The positive (and negative) predictive value of the test is however dependent on the prevalence of disease in the population

    In assessing the usefulness of a test, the most valuable parameter is the lielihood ratio; that is, the ratio of the probability of a patient with the disease having a significant result divided by the probability of having the same result when the patient is healthy. This is calculated as Sensitivity/(1-Specificity). A high likelihood ratio indicates a useful test (32, 33).
12. Truncation errors: Artefactual truncation error can arise from strongly correlated variables. Castilla and Paz (34) present an example of truncation in Down's Risk Screening occurring because of the strong correlation between paternal age and parity.
13. Failing to define whether a significance test was one or two-sided: There may be a temptation to use a one-sided test rather than the two-sided version because the probability level is lower, and therefore the apparent significance seems greater – particularly when the direction of all the data is consistent. In general, all significance tests should be two-sided (35).
14. Confusing standard error with standard deviation: Standard deviation is a descriptive index measuring variability between individuals in the factor being investigated. The standard error is a measure of uncertainty in a sample statistic (e.g. the *standard error of the y estimate* in a linear regression). The standard deviation is relevant when variability between individuals is of interest, while the standard error is relevant to summary statistics such as means, proportions, slopes, etc (31).

Errors in interpretation

An important statistical distinction is the difference between statistical and clincal significance. A result may be statistically significant, yet the difference may not be of any practical importance because the effect is too small or irrelevant. Conversely, there may be a clinically significant effect but the investigation fails to reveal it, perhaps because the study was too small or there was excessive random variation. A related problem is misunderstanding of the significance of a result. Take for example the concept of a reference interval, which may lead to the inference that subjects whose values fall outside the interval are abnormal. While this may be true, such an inference is not valid, both because the interval by definition excludes a fixed small percentage of healthy subjects, and also because the values of the variable in ill subjects are not known. These are errors of interpretation.

Erroneous interpretations of 'significant' and 'not significant' P values abound. Many believe that the goal of research is a significant result, inferring that a non-significant result implies that the research was unsuccessful. This attitude is reflected in the description of study results as 'positive' and 'negative' respectively, and in the description of the latter as having "failed to reach statistical significance".

Another source of error is the belief that a statistically significant association in itself provides direct evidence of a causal relationship between the variables concerned. Causal relationships must be established by non-statistical means (except in some randomised trials). Great care must be taken in comparing variables that vary with time as it is easy to obtain spurious associations.

Errors in the presentation of results

The final presentation of results from a research project is as important as the investigation. It is on the basis of the information presented, and its format, that the research will be assessed by your peers. One obvious requirement is to state what statistical analysis has been used, yet Mosteller *et al.* (36) examined 132 controlled trials in cancer resea rch and found that the method of statistical analysis was specified in only 46 (35%). Any research paper must include a clear statement of results, which includes all data necessary to justify the conclusion. A common result presentation error is to state means (or medians) of continuous data without any indication of their variability; that is, without a standard error (37). The obvious problem here is that comparisons of means cannot be made without some knowledge of their imprecision.

Most scientific journals have published statistical guidelines for authors (30) however there are some general points which apply to the presentation of all research findings, even projects that may not be formally submitted for publication:

1. Numerical precision: Inappropriate precision impairs a research paper's readability and credibility. Although some journals may provide guidelines for numbers of significant figures, the following general rules should be used. When presenting summary statistics or the results of analyses, such as means, standard deviations and regression equations, the precision of the original data should be borne in mind. Means should not usually be quoted to more than one further decimal place than the raw data, but standard errors and standard deviations may require extra decimal places. Percentages do not need to be given to more than one decimal place at most, especially in small samples.

    Test statistics such as t and X^2 do not need to be given to more than two decimal places. Similarly, P values do not need more than one or two significant digits, and it is not necessary to be specific below, say 0.0001. However it is often

helpful to give the exact value of *P* rather than as less than a particular cut off, eg. <0.05. Altman cites many examples of unnecessary and spurious precision in published papers (9).

2. Graphical presentation: Common misleading features of graphs include:
   d) The lack of a true zero on the vertical axis;
   e) A change of scale in the middle of an axis (especially heinous in a histogram);
   f) Three-dimensional effects;
   g) Failure to show coincident points in a scatter diagram;
   h) showing a fitted regression line without a scatter diagram of the raw data;
   i) Superimposing two (or more) graphs with different vertical scales (especially when they do not start at zero); and
   j) Plotting means without any indication of variability.

Statistical problems of interest to clinical scientists

All clinical scientists should be aware that robust and readily accessible experimental designs are available for most 'trials' conducted by laboratories. Evaluation of new equipment or reagents, and the determination of reference intervals, are situations where protocols or experimental designs are available and should be used (38-40). Failure to use these models may lead to many of the problems described above. Outlined below are requirements and common pitfalls for these particular situations.

The determination of reference intervals

Determination of reference intervals is a common problem in clinical laboratories (40-42). The principle is well known; that is, to classify subjects as normal or abnormal with regard to some biochemical measurement, as an aid to clinical decision-making and subsequent treatment. If we wish the measurement itself to be a measure of abnormality, then we need to be able to describe the variation among some defined group, usually of healthy subjects. In this regard, the following should be considered:

1. Is the distribution normally distributed? There are a few very commonly seen errors in the clinical sciences. One is assuming all variables are normally distributed when calculating a reference range. The distribution of many medical quantities do not follow, or even approximate, the normal distribution.
2. Appropriate sample for determining the reference interval: In relation to the determination of reference ranges it is vital to be aware of the variables which may affect a measured analyte, and hence choose an appropriate sample. The concept of 'clinical normality' is elusive, and any definition will be specific to the context. Reference intervals are often derived from samples taken in hospital from subjects subsequently found not to be seriously ill. Factors other than disease affect many of the commonly determined biochemical analytes include: age, sex, fasting/non-fasting status, circadian rhythm, menstrual cycle, physical activity and race (41, 43).
3. Use of parametric versus non-parametric statistics: A common statistical question is whether to use a parametric method or the percentile (nonparametric) method. The percentile approach is attractive because of its simplicity and validity for all data sets, but there are two important advantages of using a parametric method based on Normal distribution theory. Firstly, the resulting confidence intervals for values defining the reference interval are much narrower than for the equivalent non-parametric method. Secondly, the use of the Normal distribution allows any subject's measurement to be expressed as a standard deviation score, and hence located at a particular percentile, which is much more informative than knowing whether they are inside or outside the reference interval. In other words, we can see how abnormal a value is. It should always be remembered that it may be possible to transform the data so that the parametric approach may be used, however the assumption that the data follows a Normal distribution should be verified.
4. Appropriate sample size for reference interval: The sample size should be large enough to restrict uncertainty about the limits of the reference interval, preferably with at least 100 subjects for a parametric analysis and 200 for the percentile method.

   In all cases, reports of new reference intervals should specify the criteria for inclusion of subjects, and the statistical methods used. Sample selection methods can be classified as *a priori*, where subjects are selected randomly from the population of interest, or a *posteriori,* where subjects are chosen from an existing result database on the basis of certain criteria. In general, sample sizes should be greater where *posteriori* techniques are used.

Method comparison studies

The comparison of a new method with an existing one is another common laboratory project and yet one that is infrequently performed correctly (44). Westgard and Hunt (45) have discussed various statistical techniques for method comparison. Table 7 summarises their findings in this regard, relating the sensitivity of common statistical methods in this area to various types of error. The data show that no one technique is immune to the random, constant and proportional errors. Rather, a collection of parmeters must be interpreted carefully if the correct overall picture is to be gleaned.

Table 7. Sensitivity of statistical parameters to different types of error

Statistical test/parameter	Type of error		
	Random	Constant	Proportional
Least squares regression:			
Slope	No	No	Yes
y intercept	No	Yes	No
Standard error	Yes	No	No
t test:			
Bias	No	Yes	Yes
Standard deviation of differences	Yes	No	Yes
Correlation coefficient	Yes	No	No

Caution must be heeded when interpreting correlation coefficient values, particularly with respect to method comparisons. The ratio of explained variation to unexplained variation is called the coefficient of determination. If there is zero explained variation, i.e. the total variation is all unexplained, then this ratio is zero. Alternatively, if there is zero unexplained variation, i.e. the total variaton is all explained, then this ratio becomes one. If there is some component of explained and unexplained variation, then the ratio lies between zero and one. This ratio, labelled as r^2 can be used to describe explained error. For example, from an r^2 of 0.75, it can be said that 75% of the variation in y is explained by variations in x.

Westgard and Hunt have pointed out that although both r and r^2 are sensitive to random error, they have no units, and thus fail to quantitate the error present. Furthermore, the correlation may vary markedly depending on the range of values covered by the data. Correlation will also fail to detect constant or proportional differences

between two methods. Therefore, whilst an extremely common and useful measure of association, these quantities are of little value as descriptive statistics for method comparison.

In the context of method comparison, linear regression is a technique commonly used and abused, for a number of reasons. Firstly, there is the common confusion of correlation and regression, already examined in detail earlier in this review.

Secondly, there are a number of linear regression methods available, each with its own assumptions, advantages and disadvantages. Classical linear regression will produce a slope and intercept for two lines – one minimising the vertical distance between the line and the data (that is, assuming there is no error in the measurement of X), the other minimising the horizontal distances (assuming that there is no error in the measurement of Y). Clearly, neither situation is likely in clinical laboratories, where both measurements will usually incorporate a component of the overall error. For this reason, a more sophisticated technique allowing for error in each method is preferable. The methods of Deming, and that of Passing and Bablock, are examples of more robust techniques. The reader is referred elsewhere for a more detailed examination of these techniques (46-49).

Thirdly, the calculation of linear regression parameters, in common with statistical techniques already discussed here, is dependant on a number of assumptions being fulfilled. These include the requirement of a linear relationship among the data, each variable should be normally distributed, and there should not be any other extraneous variables that degrade the relationship being examined. At the very least, there should be a careful examination of a plot of the data before analysis, with a careful investigation into unusual outliers, if any.

Often, careful examination of a plot of the data, together with a knowledge of the regression line parameters can be more useful than a detailed statistical analysis of other parameters such as the correlation coefficient, the standard deviation of the method differences, or the standard error of the estimate.

Replicate measurements are rarely made in method comparison studies, so that an important aspect of comparablility is often overlooked. A method with poor precision will never agree well with another method.

Why are there so many statistical errors in published papers?

Mistakes in published papers are probably due to an inadequate understanding of statistics by those using the methods, which in turn derives from inadequate statistical education. Undergraduate teaching of statistics should introduce some of the key statistical concepts, but does not provide adequate understanding and experience in deciding which test to apply in a particular situation. Several studies have shown that the statistical understanding by doctors of basic statistical methods and ideas is inadequate (50). Consequences of the inappropriate use of statistics include the following:

1. Other scientists may be led to follow false lines of investigations;
2. Future patients may receive an inferior treatment, either as a direct consquence of the results of the study or possibly by the delay in the introduction of a truly effective treatment; and
3. If the results go unchallenged the researchers may use the same inferior statistical methods in future research, and others may copy them.

Altman and Bland (50) have considered reasons for the widespread misuse of statistics, and highlight the effects of misleading textbooks and easily accessible computer programs. The increased availability of computers and powerful statistical software has given wide access to complex analytical methods, but there has not been an accompanying increase in the understanding of those techniques. Statistical tests of significance are therefore easy to apply, and, with the advent of computers, the results of almost every possible analysis can be performed and presented ot the eager researcher. This can unfortunately lead to the situation where an investigator may be tempted to allow his statistical analysis to generate his hypotheses. The philosophy underlying this approach is to measure everything, test the results for each measurement by computer, and then pick out all those items that achieve statistical significance.

When evaluating the strength of an argument for or against some treatment or scientific hypothesis, what whould you look for? The investigator should clearly state;

k) The hypothesis being examined (preferably, as the specific nul hypothesis to be analysed statistically);
l) The data used to test this hypothesis and the procedure used to collect them (including the randomisation procedure);
m) The population the samples represent; and
n) The statistical procedure used to evaluate the data and reach conclusions.

Conclusion

Modern computer technology has provided scientists with the tools to easily apply statistical tests to any form of data. Inadequate education and understanding has led to a general 'intimidation' of scientists in the area of statistical methods, particular in the process of identifying suitable test methods. These factors, and others, have created an environment where an alarming number of scientific papers utilise inappropriate statistical methods.

This need not be the case. An improved awareness of proper research design, basic statistical techniques, and the assumptions upon which they are founded, should provide for more effective and easier to read papers in the scientific literature.

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Editor's note

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This article will be required reading for all NZIMLS Fellowship candidates subject to ratification by Council of the NZIMLS.

Letter to the Editor

A Comparison of the DiaMed Gel Technique with Conventional Tube Techniques in a Transfusion Medicine Laboratory

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The purpose of the trial was to compare the performance of the DiaMed Gel technique with our traditional tube techniques by challenging them with selected samples. We were aiming to see if the DiaMed system could reduce our workload or eliminate the anomalous results sometimes seen in our tube technique. A secondary purpose of the trial was to give our staff hands-on experience of using this technology.

We tested samples with known antibodies, samples which gave anomalous results when tested by tube techniques, and routine samples. All the samples were tested in duplicate using the gel and tube techniques.

The results were varied. It appears that there are advantages and disadvantages associated with both techniques. Many more samples would need to be tested to draw any significant conclusions.

Introduction

At present we use a tube technique for all our patient blood grouping and antibody screens. The techniques used for antibody screening are two stage enzyme and low ionic strength indirect antiglobulin technique (LISS IAT).

DiaMed offered us the opportunity to trial their system in our laboratory. The DiaMed system comprised an electronic multichannel pipette, DiaMed card centrifuge, 37°C incubator, automated reader and a collection of DiaMed gel cards. For several weeks we used the system in parallel with our current techniques using selected samples chosen to best illustrate any differences.

Testing and Results

1) Grouping:–

i) We found no differences between the results of the DiaMed grouping cards and the tube technique during routine ABO and Rh grouping.

As well as normal samples we tested the following:

ii) Weak D – The weak D cell from the in-house panel gave good reactions on both the DiaMed grouping and DiaMed D typing cards. It was identified by the reader as a weak D (D^u) and could be visibly determined as a weaker D also.

iii) Mixed Fields – We tested two samples which had given good mixed field results by tube technique. One was a cord blood contaminated with maternal blood and the other was a post transfusion Rh phenotype. Both of these samples gave easily identifiable mixed field reactions with DiaMed.

iv) A patient with leukaemia with a depressed expression of A antigen. The DiaMed system detected the weak expression of the antigen and showed a mixed field result on the grouping card, a result also seen by tube technique.

The DiaMed grouping cards did not show any advantage over the tube technique for routine blood grouping in terms of sensitivity or ease of reading. The longer time needed for DiaMed grouping could be perceived as a disadvantage. The results seen with the above samples were the same as those seen using the tube technique.

2) Red Cell Antibody Screening:–

We selected a variety of samples to test using the DiaMed system, to determine if the DiaMed would eliminate some of the false positive screens we were finding by our tube technique and also to ensure that the DiaMed system would detect significant antibodies we had detected by tube.

i) Rouleaux – One sample, which contained no antibodies but had exhibited moderate rouleaux by tube enzyme technique, gave a negative screen when tested by DiaMed. This was consistent with DiaMed claims that the system eliminates the interference caused by rouleaux in enzyme techniques. However more samples would need to be tested to confirm this finding.

ii) Indeterminate antibodies – Two of the samples tested had given positive results in the tube antibody screens (one in enzyme and the other weakly in enzyme and IAT) but no antibody could be determined upon further investigation. Both of these samples gave negative screens, both enzyme and IAT, when tested using DiaMed.

iii) Enzyme panagglutinin – One sample with a weak enzyme panagglutinin by tube technique gave a negative screen when tested by DiaMed.

iv) Anti-K – One sample which contained an anti-K had been found to be reactive in IAT but not enzyme by tube technique. When tested using the DiaMed system it reacted in both IAT and enzyme tests.

v) LISS-dependent antibody – One of the samples with a non-specific LISS dependent antibody by the tube technique gave a negative antibody screen when tested using DiaMed.

The DiaMed system appeared to be less prone to false positive results and "nuisance" antibodies than the tube technique, particularly in regard to rouleaux and enzyme panagglutinins. This would lead to fewer investigations and full crossmatches if used for routine antibody screens. However, we tested a low number of samples and more comprehensive testing of the system would be required to substantiate this.

3) Antibody Identification Panels:–

i) Anti-D-One sample contained weak anti-D reacting in enzyme but not IAT by tube technique. When panelled using the DiaMed system, anti-D was clearly identified by both enzyme and IAT techniques.

ii) Anti-Yk^a-A sample from a patient whose serum contained Anti-Yk^a reacted with all cells except panel cell 3 (presumably Yk^a negative) when tested by DiaMed. When tested by tube technique four of the panel cells failed to react, and some of the reactions were weaker than that seen when tested using DiaMed. This would imply that the DiaMed system was detecting the weaker expressions of the antigen which were not being detected by tube technique. In this instance the less sensitive tube technique was an advantage as it allowed us to find adequate numbers of crossmatch compatible units to this clinically insignificant antibody.

iii) Warm reacting autoantibody-A sample from a patient with warm autoimmune haemolytic anaemia (WAIHA) gave similar reactions by DiaMed as by tube technique.

iv) Eluates-We were also keen to determine how the DiaMed system would cope with the testing of eluates, which are usually heavily stained by free haemoglobin. We tested a heavily stained eluate which was unreactive by tube technique and it was also unreactive by the DiaMed technique. In order to determine whether the DiaMed system could detect a positive result in an eluate we added a mixture of anti-D and anti-C to the unreactive eluate and tested it using the DiaMed

system. Even though the sample was heavily stained with free haemoglobin, both the anti-D and anti-C were easily identifiable.

v) Indeterminate reactions – A sample from a trauma patient reacted weakly in both the IAT and enzyme screens by tube technique, however no antibody was identifiable upon further investigation. We tested the sample using the DiaMed system in the hope that it may clarify the screen results obtained by tube. Surprisingly, by DiaMed the enzyme panel was completely negative but the IAT panel gave weak results consistent with an anti-D pattern, with cell 3 being noticeably stronger. We received a repeat specimen from the patient and again the tube antibody screen was positive, but this time only in enzyme, with an enzyme panagglutinin identified. When the repeat sample was tested using DiaMed the enzyme panel was again negative, but the IAT panel reacted only in cell 3 and showed no sign of the anti-D pattern previously seen.

vi) Rh antibodies – A sample contained anti-E (reactive by enzyme only in the tube technique) and a probable anti-c which was no longer reliably detectable by tube technique. When tested using the DiaMed system, both the IAT and enzyme panels were repeatedly unreactive.

It is difficult to determine if the DiaMed system has any advantages over the tube technique in antibody investigation, due to the small number of specimens tested. The DiaMed system appears to be more sensitive to Rh antibodies by IAT technique, in some instances but not others.

vii)NIPS survey:–

A recent NIPS survey involved a patient whose serum contained a combination of anti-C, anti-D and anti-E, with the anti-E being detectable only by enzyme by the tube technique. Of the three units to be crossmatched, two were rr and one r''r.

When tested by the DiaMed system the anti-E was also detectable (quite strongly) by IAT technique, however it failed to react with the r''r cell in the DiaMed IAT crossmatch. Because of this anomaly we did some further testing. We tested 3 r''r cells in duplicate against the NIPS serum with the following results:

	NIPS serum containing	anti-C, anti-D and anti-E
NIPS r''r	? (indeterminate result)	1+
DiaMed Panel 5 r''r	2+	2+
In-house panel r''r	-	-

To standardise the test we stored all three samples in the DiaMed CellStab preservative solution for a few days and re-tested the samples, with identical results. We phenotyped the DiaMed panel 5 r''r cell to ensure it was really that phenotype as it was giving consistently stronger reactions than the other two r''r cells. The DiaMed cell was a r''r. We then tested a variety of r''r cells against the NIPS serum to try to establish what was causing the anomalous results.

The DiaMed panel 5 and DiaMed QC r''r cells both reacted strongly giving 2+ reactions. One local r''r donor unit also gave a 2+ reaction. The NIPS r''r, our in-house panel r''r and another donor unit r''r all gave very weak/negative reactions. We are still no closer to determining the cause of the variation in reaction strengths.

4) Direct Antiglobulin Tests (DATS):–

i)We tested cord blood samples from two babies with ABO haemolytic disease of the newborn (HDN), which had positive DATS by tube technique of 1+ and 2+ respectively. The DiaMed system interpreted the groups correctly and gave a 2+ positive reaction in both DATS.

ii)The DAT on a sample from the patient with the LISS dependent antibody was positive when tested by DiaMed. (The antibody screen for this patient was negative when tested by DiaMed.)

This very small number of samples tested is not a comprehensive evaluation of DiaMed system's performance in the testing of DATS, but revealed no anomalies.

Conclusion

We found the DiaMed system to be user friendly and "tidy" to use, but some of the results mentioned above seem to indicate that it is not the "cure to all ills" of antibody detection and identification and can give anomalous results with some samples. I think it is fair to say that the number of anomalous or false positive results due to rouleaux, enzyme panagglutinins or positive antibody screens which are negative upon investigation, is reduced when using the DiaMed system. Two of the samples tested indicated that the DiaMed IAT technique may be more sensitive than the tube IAT technique that we are currently using. Whether it is sensitive enough to allow us to discontinue using enzyme antibody screens can not be determined without testing a larger number of samples.

Care has to be taken when using the system, especially in pipetting, reading of results and making correct cell suspensions to ensure correct results.

Before evaluating this system I was under the impression that all the results would be very "clear-cut". However, it became apparent that the system also gave weak results, which needed very careful reading. Some of the reactions were weak enough to be missed, even when careful reading was employed, but were detected by the automatic reader. More evaluation is needed to ascertain whether these very weak reactions are clinically significant. Mixed field results were fairly easily identifiable using this system with the two cell populations clearly defined. I found it very useful to have the automated reader during the trial, as it gave us an opportunity to compare our manual reading with that of the reader, and gave us a good idea about if we were over or under reading the cards by eye.

From our limited experience in this small trial of the DiaMed system, there seems to be no advantage in using it for routine ABO and Rh grouping; the extra cost and longer test time are not balanced by any perceptible benefit.

The use of the DiaMed system for performing routine antibody screens would appear to have the advantage of detecting fewer false positive screens, especially those due to misinterpreted rouleaux and enzyme panagglutinins which would result in fewer identification panels and full crossmatches being performed. Whether this benefit would off-set the additional expense would need to be further evaluated.

We are grateful to DiaMed for giving us the opportunity to gain experience with the gel technology, although to date we have no plans to change our routine techniques.

COUNCIL NEWS

MAY 1999

Our Website - Launched at the South Pacific Congress

www.nzimls.org.nz

Membership information, links with education bodies, Council and SIG contacts and the annual calendar of activities and Continuing Education opportunities will be kept up-to-date on our website. For input requests, please contact the webmasters

Chris Kendrick (Council) fax 06 350 5637
Fran van Til (EO) fax 03 313 2098

MOLS

The MLTB MOLS programme is nearing the end of its pilot period.

The Council of the NZIMLS has resolved that the President write to the MLTB:

> "The president has written to the MLTB proposing that their MOLS Programme be succeeded by a similar continuing development programme to be administered by the NZIMLS. Audit responsibility would remain with the MLTB."

Council has requested an answer by 30th September 1999.

If accepted draft proposals will be circulated via SIGs for comment.

Master Medical Laboratory Science (M.M.L.S.) - Massey University

Start: 2000
See the prescription advertised in this Journal

Subscription Payment Options

These have been evaluated over the previous year for three key reasons:

1. NZIMLS is losing $ processing fees to a significant number of employers, ie your fortnightly deduction is further deducted by your employer before being forwarded to your professional body as authorised.
2. The NZIMLS faces annual cashflow management challenges.
3. The NZIMLS auditors are challenging the level of unfinancial members and their costs to financial members.

Payment options and recommendations will be forwarded to all members over the coming months.

Worldwide Biomedical Science Day

Saturday 15th April 2000

Theme for 2000 - set by WHO - Laboratory Technology Unit

"Medical Laboratory Science
The key to the Diagnosis of Communicable Diseases"
Spotlight on "Sexually Transmitted Diseases"

Ideas for Actions:

- Write an article on the contribution that your speciality makes towards the diagnosis and control of STDs.

  Publish in our Scientific Journal NZIMLS.

- Use the day to promote your laboratory locally using the International Day theme eg diagnosis of STDs.

- Via: posters, display boards
  open lectures, seminars
  discussions with other health professionals highlighting the theme and expertise of medical laboratory scientists

- Can you get the Press to cover your promotion?

NZIMLS 2000

Sun Surf Sulphur Scientific Session in the Bay of Plenty,
16-19 August 2000, Rotorua Convention Centre.

The first Annual Scientific Meeting of the third millennium will be all you want it to be:

fascinating facts	*fantastic fishing*
strategies for science	*sensational scenery*
friendly informative forums	*fun and adventure*

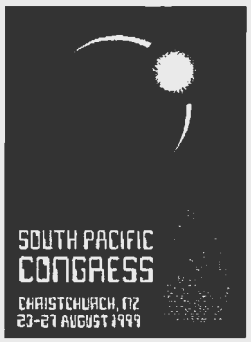
This is your professional opportunity for dialogue with colleagues in the diagnostic delivery services and commercial suppliers of technologies and consumables.

Theme for the change of the millennium is Paradigm Shifts.

As knowledge expands and health dollars shrink, as disciplines merge or specialise more, as the public expectations of profession performance is higher than ever before. Plan now for MOLS points at the BOP Conference 2000.

Conference Management
Fran van Til, Executive Events
P O Box 647, Rangiora, New Zealand
Tel: +64 3 313 2097 Fax: +64 3 313 2098
Email: fran @exevents.co.nz Internet: www.exevents.co.nz

SOUTH PACIFIC CONGRESS



To the Organising Committee
5th South Pacific Congress
Christchurch 1999

On behalf of the NZIMLS membership and Council

Thank you Thank you

Thank you for your hard work
professionalism
commitment and dedication

to produce an unparalleled scientific programme. Inviting a NASA astronaut to present life and their scientific research in space was truly inspirational, but well balanced by practical, fully applicable workshops and dynamic speakers in all disciplines.

The social programme was tremendous fun, and all delegates welcomed the opportunity to unwind from the concentrations required for the scientific topics of the day. Fun and laughter on mystery events or going to a masquerade ball are as necessary as academia.

The venue placement and presentation was ideal for everything from scientific fora to the Industry Display as well as allowing delegates to socialise and network.

The PCO team of Executive Events provided a friendly, effective service which delegates and guests most appreciated.

The entire Congress was of an international standard and a credit to you all. Thank you.

Equally important, the membership and Council of the NZIMLS thank our colleagues in the commercial sector for their tremendous support of our annual scientific meeting.

The Industry Display of technologies, consumables and services is an important feature of our annual scientific meeting. It offers many delegates their only opportunity to examine these options first hand.

Display stands presented many and varied wares and many also gave away pens, pads, rulers, chocolates and various samples.

Innovative offers are always a feature and worthy of mention. This year of particular note were SCIANZ/Bayer Digital Camera competition and the masseuse on the Bio-Rad stand.

The Industry must also be acknowledged and thanked for its financial support of other areas of Congress that keep delegate fees as low as possible. For example, many fora were sponsored by many different companies, Abbott sponsored the Icebreaker, Biolab the name badges, and Roche Diagnostics, the wine at the Congress Masquerade Ball. The Platinum sponsor was Dade Behring.

Sponsorship is not limited to our commercial colleagues. Major sponsors included SGS for the satchels and Canterbury Health Laboratories for the opening ceremony.

Everything must be budgeted for in any organisation and we the diagnostic delegates thank you for your generosity to us the product-end users and your continued commitment to our profession and the NZIMLS.

CONGRESS REVISITED

Life Members

Two members were awarded life members of the Institute at the 54th Annual General Meeting of the NZIMLS held at the South Pacific Congress. Life membership certificates were presented by the President, Shirley Gainsford.

Warren Dellow
Med-bio Limited

Paul McLeod
Nelson Hospital

Jim Le Grice Icebreaker

(Sponsored by Abbott Diagnostics)

Winner of the Industry Display Award
Clinical Data

Brian Hanrahan
Abbott Diagnostics

Tim Lester, Lifeguard promoting the BOP 2000 Conference, Rotorua

3 Wise Men - (from left)
Rob McKenzie, Ngaio Diagnostics
John Aitken, Organising Committee
Chris Kendrick, NZIMLS Council

The youngest delegate at the SPC - Daniel with his mother, Sandy Woods, Vaughan Clift and Ken Cockrell

Opening Session *(Sponsored by Canterbury Health Laboratories)*

The Cathedral Grammar School Choir

Mayor of Christchurch
Welcome to Christchurch

Katherine Brown receiving
Jennifer Castle's Fellowship Certificate *from President Shirley Gainsford*

Dr John Munro
Dade Behring Diagnostics
Platinum Sponsor

Kenneth Cockrell
Astronaut

Dr Vaughan Clift
Physician

NASA /Johnson Space Centre, Houston, Texas, USA

Our two very special keynote speakers presenting in tandem

Social Events *(Sponsored by SCIANZ/Bayer and Roche)*

International Relations!!
Kevin Ericksen AIMS President
with NZIMLS Presidents
Shirley Gainsford & Anne Paterson

Houston, we have a problem!
I am not enjoying this!!

NZIMLS President's Report 1998

The most exciting result is a net gain of $22000 for the year which is due to a successful conference and better financial management as a result of quarterly balancing of the accounts.

This surplus now allows the Institute to use a credit card system for its members to pay subscriptions and fees and to have a website which is being launched at the 1999 Annual Scientific Meeting.
A number of projects have been completed.

a) protocols for organising the Annual Scientific Meeting
b) job description for the editor of the Journal
c) guidelines for Life/Honorary membership

Because of our concern with the financial situation of the International Association of Medical Laboratory Technology (IAMLT), we obtained a legal opinion on the liability of member organisations, should the IAMLT collapse financially. The opinion was that there is no liability for the NZIMLS.

The number of candidates for the Qualified Technical Assistant examination has decreased. There were 55 candidates in 1994, 50 in 1998 and only 39 in 1999. We have however, an increase in candidates for Fellowship and one Fellowship has been awarded this year.

The Council has approved a donation of $5000 to the Para Pacific Training Centre to support the Quality Assurance programme in the Pacific Islands.

For me, the most satisfying aspect of this year has been the Special Interest Group (SIG) activities. The Biochemistry SIG was revived and all SIGs except cytology have held meetings. Thank you to SIG committee members for the major contribution you have made to the continuing education of medical laboratory personnel in New Zealand and for your personal contribution to the success of the Institute.

The most unsatisfying aspect of NZIMLS business has been the suggested changes to laboratory funding by the Health Funding Authority. We have responded to two consultation papers from the HFA. Different persons wrote the papers and there were major changes in the contents of the second paper. There is no doubt that this is a major cost cutting exercise. Our concerns are a loss of quality of service and insufficient funding for ongoing training and education of laboratory staff.

I thank all the companies who sponsored the 1998 Annual Scientific Meeting and helped to make it a success. Thankyou also to our continuous sponsors for the year:

Med Bio Limited awarded $150 for the best article published in each issue of the NZIMLS Journal.

Roche Boehringer Ltd offered an award of $1000 for the best biochemistry paper presented at the 1998 ASM. However no award was made, as in the opinion of the judges, no paper was of a sufficiently high standard.

The award of $1000 by Scianz Co ion for advancing personal development, allowed Paul Austin of Auckland Hospital to visit a laboratory in Sydney.

Biolab Scientific Limited was our major sponsor and contributed $5000 for the production of the Membership folio and inserts.

We are indebted to all these companies who continue to support our profession.

When I became President there were 3 vacancies on Council and a cash flow problem. I feel very proud that the cash flow problem is gone and that we have a good team beginning new projects to enhance the service to our members.
I feel privileged to have worked with Council and SIG members who are so committed to the profession.

Shirley Gainsford
August 1999

NEW ZEALAND INSTITUTE OF MEDICAL LABORATORY SCIENCE (INC)

Minutes of the 55th Annual General Meeting Held at the Christchurch Convention Centre, on Wednesday 25th August 1999

Chairman

The President (Ms S Gainsford) presided over the attendance of approximately 36 members.

Apologies

Motion:
Moved T Mace, seconded R Bluck
That the following apology be accepted:
S Benson

Proxies

A list of 2 proxies were read by the Secretary.

Minutes

Motion:
Moved J Sheard, seconded T Mace
That the Minutes of the 54th Annual General Meeting held on Wednesday 2nd September 1998 be taken as read and accepted as a true and correct record.

Business Arising

Council to follow-up on a venue for the Year 2000 conference. This is to be held in Rotorua and will be the Bay of Plenty 2000 Conference.

Remits

Motion:
Moved T Rollinson, seconded R Siebers
That Policy Decision Number 3 be changed from.

Policy Decision No 3 (1972): Council will make and administer awards to members of the institute, the details of each award will be recorded and may be amended from time to time by resolution of Council. The Summary these details shall be published annually in the Newsletter.

TO

Policy Decision No 3 (1972): Council will make and administer awards to members of the Institute, the details of each award will be recorded and may be amended from time to time by resolution of Council. The summary of these details shall be published annually in the Journal.

Explanation:
The NZIMLS does not produce a newsletter.

Carried

Motion:
Moved T Rollinson, seconded A Paterson

That Policy Decision Number 5 be changed from:

Policy Decision No 5 (1978): That medical supply companies should not be approached to aid in the finance of Branch meetings; companies may be invited to Regional Seminars and although donations may be accepted money is not to be solicited.

TO

Policy Decision No 5 (1978): That medical supply companies should not be approached to aid in the finance of Branch or Special Interest Group meetings; companies may be invited to Regional Seminars and although donations may be accepted money is not to be solicited.

EXPLANATION

(a) The ASM is the appropriate venue for an industry display.
(b) Company representatives have told Council that they wish to attend SIG meetings on an informal basis.

A Paterson spoke to the motiion. Council have been looking at the SIG meetings and conference and want both to be complimentary to each other. Industry have indicated that they want to attend SIG meetings on an informal basis.

Carried

Policy Decision No 1 (1974): That all committees and meetings convened under the auspices of the New Zealand Institute of Medical Laboratory Science (be subject ndar reference of parliamentary procedure and that Guide to Meetings and Organisations' by Renton.

Motion:
Moved T Rollinson, seconded R Siebers
That Policy Decision Number 2 be reaffirmed.

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

Carried

Presidents Report

Motion:
Moved S Gainsford, seconded A Paterson
That the President's Report be received.

Annual Report

Motion:
Moved A Paterson, seconded R Siebers
That the Annual Report be received and adopted.

Financial Report

Moved T Rollinson, seconded R Anderson
That the Financial Report be received and adopted.

Election of Officers

The following members of Council were elected unopposed:

President	A Paterson
Vice President	L Milligan
Secretary/Treasurer	T Rollinson
Region 1 Representative	S Benson
Region 3 Representative	C Kendrick
Region 4 Representative	G Moore
Region 5 Representative	S Baird

Awards

The award winners were announced and the awards where possible were presented by the President:

Life Membership of the NZIMLS

Warren Dellow	Med-bio Limited
Paul McLeod	Nelson Hospital

Qualified Technical Assistant Awards

Clinical Biochemistry	Sara Thomas, Medlab South
Medical Cytology	Regan Kendrick, Diagnostic Laboratory
Haematology	Autonina Volikova, Diagnostic Laboratory
Histology	Bharati Cheerala, Diagnostic Laboratory
Immunology	Marita Smit, Canterbury Health Laboratories
Microbiology	Deborah Wetherall, Taranaki Medlab Marie Hogg, Medlab Timaru Donna Doreen, Medlab Central
Mortuary Hygiene & Technique	Robert Peters, Auckland Hospital

Honoraria

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

Auditor

Motion:
Moved T Rollinson, seconded C Kendrick
That Hillson, Fagerlund and Keyse be appointed as the Institute's auditors.

General Business

A Paterson advised of the death of a colleague at Whakatane Hospital, Karen Hastie.

Mike Lynch, PPTC thanked and acknowledged the continued support from NZIMLS during the year.

Questioned why the Council sought a legal opinion as to the NZIMLS's liability should the IAMLT become unfiniancial? The legal opinion could not be certain but felt that the NZIMLS would not be liable. If the IAMLT executive was held liable then it could fall back on the NZIMLS. Noted that the IAMLT are addressing their costs, in particular the cost of running their office.

Questioned if the NZIMLS was looking to offer an examination for registration? Is there any negotiation for these people to have a shortened degree course to achieve qualification on top of a BSc? Noted that people knew for some time that the examination avenue would not be available. Paul McLeod advised that the Board is aware of this situation and that they do not want to block people either. There is a difficulty with people who do not have a board-based qualification.

Venue for the Year 2001 Annual Scientific Meeting

No venue was confirmed. Council will follow-up.

Meeting closed at 5.25pm.

Life Membership

Life membership of the Institute is awarded to any member who Council considers has given outstanding service to the Institute and profession of medical laboratory science.

This year a number of nominations for life membership were received from the Special Interest Groups and Council.

The Council voted to bestow this award on two members.

Shirley Gainsford, President NZIMLS, made the awards at the Annual General Meeting of the NZIMLS on Wednesday 25th August 1999 and included the following speech.

Warren Dellow

Warren started training as a medical laboratory technologist in 1973 at Nelson Hospital. He was in one of the first groups to undertake NZCS at polytechnic – a change from in-house training. Warren gained Part II and Part III in Biochemistry, and qualified in 1979. He was then at Pearson Laboratory in Christchurch where he became 2IC biochemistry.

Warren then made a change from laboratory work and went to work for Ebos Ltd, one of the supply companies. He subsequently decided to start his own company along with David McDonald and in 1983 Med-Bio limited came into existence.

Warren has been a major supporter of the NZIMLS all his working life.

He has attended most NZIMLS conferences since 1978 and in 1988 he and John Aitken revived the South Island Seminar, with a meeting at Mariua Springs in the Lewis Pass. He has been involved with the organisation of the SI seminar ever since and offers a travel prize for best presentation at this seminar.

Since 1993 Med-Bio has sponsored a prize for the best original paper in each issue of the NZIMLS journal.

Warren is still involved with technical laboratory work. He and Dr Lance Jennings are investigating the relationship between respiratory infection and acute mountain sickness using immunofluorescent assays and PCR techniques.

He also spends 2-3 weeks a year in the laboratory at National Hospital Samoa. This is primarily for business reasons but while there he helps with training of staff and fixing machines.

Warren is now a successful businessman but he is first and foremost a medical laboratory scientist who is proud of his profession and encourages others to be the same.

He has gained his success through hard work and enterprise and is an example of what is possible in our profession.

Warren also gives something back to his profession (not Med-Bio but Warren personally). He deserves recognition for the time and effort he has given on our behalf. It therefore gives me great pleasure to bestow life membership of the New Zealand Institute of Medical Laboratory Science on him.

Paul McLeod

Paul started his training in 1967 at Nelson Hospital Laboratory. He completed his training at Wellington Hospital in 1972, gaining Part III microbiology.

Paul then travelled overseas and when he returned went back to Nelson Hospital to be in charge of Microbiology where he remains.

Paul was elected to the Council of the NZIMLS as regional representative for the top half South Island in 1981. His forte became industrial relations and Paul was a chief negotiator during the industrial unrest in 1985 when laboratory workers went on strike. He remained as union delegate during the formation of the Laboratory Workers Union and then decided to concentrate on the Institute, becoming the Vice President in 1987.

Paul became President of the NZIMLS in 1990, his chief involvement at the time being to achieve the goal of a degree qualification for medical laboratory scientists. This goal was achieved twice over with the introduction of the BMLS at Otago University and Massey University.

After his term as President Paul became Treasurer of the NZIMLS and stayed on the Council until 1996. Paul has therefore served the NZIMLS for 15 years.

He has still not given up service to his profession as he became appointed to the Medical Laboratory Technologists Board in 1992 and is now the chairman of the Board.

Paul is meticulous with his preparation for meetings and shows great determination to get things done. When the BMLS at Otago Universtity became a strong possibility, Paul toured the country promoting the degree to the profession and was quite determined that it should supercede the National Diploma of Medical Laboratory Science.

His personality and good sense of humour make him one of our best ambassadors and I believe that this honour is well deserved.

Congratulations to both of you for an honour well deserved.

Shirley Gainsford

President 1996-1999 New Zealand Institute of Medical Laboratory Science

Shirley is a committed professional. She has been active in, continues to participate in and contributes to both her profession and her professional body. In the 50th year of the NZIMLS, Shirley assumed the role of President. She is the first woman President. Shirley commenced her career in 1963 at Wairau Hospital Laboratory in Blenheim under the tutorship of Hugh Bloore, the seventh President of the then named 'New Zealand Institute of Medical Laboratory Technology'. She passed her Intermediate Examination at Wairau Hospital and continued her training in Wellington Hospital Laboratory. Her father in the Air Force, was transferred and Shirley moved with her family to Wellington. By the end of 1967, Shirley had gained her 'O' level in both Haematology and Microbiology.

The OE beckoned and she spent the next 18 months travelling to London where she worked in a National Heart Hospital before returning home via Middle Eastern Europe.

On her return to New Zealand, Shirley worked at Seddon Laboratory 1969-1972, prior to its closure. During this time she gained her 'A' level in Microbiology. 1972-1988 saw her continue her studies at Massey University in Genetics while running a major laboratory section at Wellington Hospital. She was also the specialist responsible for all Mycology and STD work for the laboratory.

In 1989 Shirley took the Charge Scientist-Microbiology position at Valley Diagnostic Laboratory in Lower Hutt, where she works today. She completed a Diploma in Business Studies at Massey University in 1991.

Having been involved at a local level in the Wellington Branch of the Institute, Shirley's strong interest in education resulted in her standing for and being elected to Council as the Wellington Regional Representative in 1987. She undertook the newly combined role of Secretary/Treasurer in 1991 and worked closely with the recently appointed Executive Officer who had been employed only the year before. She chaired the newly formed Microbiology Special Interest Group when it formed in 1990 until 1995.

Shirley progressed to the Vice-President position in 1993 – only the second woman to do so.

Taking up the reins of the Institute in 1996, as President, Shirley was immediately forced to address cashflow problems. While other tasks were probably more appealing and professionally rewarding, Shirley led the Council team with a strong clear direction of fiscal responsibility of members' fees. Shirley has travelled to attend as many SIG meetings as possible promoting the Institute.

Shirley has maintained a strong scientific focus and has presented many papers at seminars and conferences. She continues to exmamine and/or moderate at all professional levels of our professional qualifications – from QTA to Fellowship.

Shirley has supported and encouraged the Fellowship Committee of Trevor Rollinson, Rob Siebers and Les Milligan to revise the Fellowship qualification – the highest academic award of the institute. On completion, she has actively promoted it to the membership. The new Fellowship options are proving popular.

Although Shirley has retired from the Council, she continues to contribute through membership of the Continuing Professional Education sub-committee of Council.

One can only say 'what a wonderful role model'. Thank you Shirley.

Personal Profile

Shirley is the eldest of her family and maintains close contact with her four sisters and three brothers, and her many nieces and nephews.

Shirley is married to Douglas Black with whom she has planned and supervised the complete renovation of their lakeside house in Rotorua. She is a keen bridge player, an even keener supporter of the Wellington rugby teams. Other recreational pursuits include the theatre, good wine, playing golf and fishing.

A window of opportunity for you ends soon! ! !

FELLOWSHIP BY EXAMINATION

Medical Laboratory Scientists who hold a NZIMLS or NZIMLT Specialist Certificate will be EXEMPT from sitting Part I Examination, IF they apply prior to:

31st MARCH 2000
This exemption will not be extended.

Successful completion of Part II - the Dissertation, will fulfil the requirements of Fellowship by this route.

Please see over for all options by which to gain Fellowship.

Fellowship of the New Zealand Institute of Medical Laboratory Science is the highest academic category of membership and carries the right to use the letters FNZIMLS.

Biolab Scientific kindly sponsors this page for insertion into your Membership Folio

FELLOWSHIP

of the New Zealand Institute of
Medical Laboratory Science

May be gained by:

Examination
Thesis
Publications

Option A: By Examination

The examination will consist of two parts:

Part I Two written papers each of three hours duration.

Part II A dissertation of 3000 - 5000 words (upon successfully attaining Part I).

Note: Clauses 3.12 & 3.13 of the Regulations:

3.12 Medical Laboratory Scientists who hold a Specialist Certificate are exempt from sitting the Part I examination. This clause will be effective for a maximum period of three years after adoption of these regulations.

3.13 The final date for applications under Clause 3.12 will be March 31st 2000.

Option B: By Thesis

This must be the original work of the candidate and not exceed 20,000 words.

Option C: By Publication

A publication summary or review of 3000 - 5000 words presenting a minimum of 5 articles published in International or Discipline acknowledged Journals. The applicant will be an author or significant contributor to these articles.

For a copy of the full Regulations and Instructions for Fellowship, please contact:

NZIMLS Executive Office
P O Box 3270, Christchurch
Tel: 03 313 4761 Fax 03 313 2098

FELLOWSHIP WORKSHOP

PRELIMINARY ANNOUNCEMENT

FOR ALL PROSPECTIVE FELLOWSHIP CANDIDATES

- **Examination - Part one**
- **Examination - Dissertation**
- **Thesis**
- **Publications**

TIME : Saturday and Sunday 1ST and 2ND April 2000

PLACE: Wellington School of Medicine

COST: $50-00

Contact the **Executive Officer**
NZIMLS
P.O. Box 3270
Christchurch

FOR REGISTRATION OF INTEREST

MSc in Medical Laboratory Science
Massey University

The MSc in Medical Laboratory Science will be offered for the first time in 2000. This degree, which is equivalent to one year of full time study, is designed for professional Medical Laboratory Scientists working full time and interested in advancement within health-related areas. It is expected that all candidates will be part time and take three of four years to complete the degree. It will be offered in block mode. Course material will be mailed out to candidates who will be required to come to the Palmerston North campus for two sessions, each lasting three days, for each paper.

Entry

The candidate will have qualified to be a registered Medical Laboratory Scientist and also have:

a) qualified for the degree of Bachelor of Medical Laboratory Science (BMLS) with ≥B-grade point average; or
b) qualified for the Massey University Diploma in Medical Laboratory Science with ≥B-grade point average; or
c) completed any other degree from a New Zealand university with ≥B-Grade point average; or
d) been admitted with equivalent status as entitled to proceed to the specified degree.

Note: The grade point average will be based on the grades for the 300- and/or 400-level papers.

The Course

A candidate shall follow, for not less than two semesters, a course of study comprising the following:

162.789	Research Project	25 points
194.792	Current Topics in Medical Laboratory Science	25 points
143.796	Quality Management for Medical Laboratories	25 points
152.742	Health Systems Management	25 points

Notes:

(a) Not all papers may be available in any one year.
(b) The research project paper will involve the investigation of an aspect of one of the subjects: Clinical Biochemistry, Microbiology, Virology, Haematology, Transfusion Science, Immunology, Histological Technique or Cytology. This can be conducted in the laboratory in which the candidate is employed. There will also be a compulsory section on research methods and communication in general.

Teaching/Delivery Methods

The MSc in Medical Laboratory Science will be offered internally in block mode at the Turitea campus, Palmerston North. The Health Systems Management paper, 152.742, will also be offered in block mode from the Albany campus, Christchurch and the Turitea campus, Palmerston North. It will be available every year. Current Topics in Medical Laboratory Science and Quality Management for Medical Laboratories will be offered in alternate years from Palmerston North, starting with Current Topics in 2000. The Research Project can be done at any stage but it is recommended that candidates discuss this with the Academic Director, Medical Laboratory Science because the project must be completed within one academic year.

For 194.792 Current Topics in Medical Laboratory Science, candidates will be supplied with Study Guides at the beginning of the academic year. They will also be required to attend two three day sessions on the Palmerston North campus, from Thursday 25 to Saturday 27 May, 2000 and Thursday 5 October to Saturday 7 October, 2000.

Note: For a 25 point paper, students are expected to devote approximately twelve and a half hours per week to that paper or 375 hours for the academic year. This 375 hours does include the 50 hours of 'on campus' courses.

Prescriptions for Papers

194.782 Current Topics in Medical Laboratory Science 25 points
An advanced course of study based on current literature of Medical Laboratory Science selected from Clinical Biochemistry, Microbiology, Virology, Haematology, Transfusion Science, Immunology, Histological Technique and Cytology. Candidates will be expected to cover areas of Medical Laboratory Science including both their current specialist discipline(s) and other areas.

143.796 Quality Management for Medical Laboratories 25 points
Principles of Quality management using a management systems approach. Total quality and continuous improvement as it relates to the delivery of services. Application of the New Zealand Code of Good Practice and other documents relevant to the operation of medical laboratories, with particular emphasis on the validation of test methods, calibration and an understanding of repeatability and reproducibility. Quality systems management in the medical laboratory with particular reference to specific codes of good practice related to Medical Laboratory Science. The above topics will be set in the context of the basic principles of quality management.

152.742 Health Systems Management 25 points
The health care systems of New Zealand and selected nations are compared and analysed in the light of sociocultural, political and environmental factors. Emergent issues for managing health services are critically examined and applied.

162.789 Research Project
Research projects will be largely medical laboratory based so there will be significant input from the individuals involved. Such research can range from comparisons of methods, evaluation of test results and patient treatment/outcome to development of new tests.

We have expertise in all areas of Medical Laboratory Science, some of which are listed below.

Clinical Biochemistry: lipid metabolism and antioxidants; ethanol metabolism; Medical Microbiology: molecular typing of *Campylobacter* species, *Pseudomonas aeruginosa* and *Candida albicans;* drug resistance in *C. albicans;* pathogenesis of *Helicobactoer pylori* and viral diseases; secreted proteins of *Yersinia enterocolitica;* epidemiology of parasites; Haematology: comparative haematology and haemostasis; factor VIII; Transfusion Science: screening tests for *Yersinia enterocolitica* to eliminate transfusion-related endotoxaemia; Immunology: antimicrobial peptides, human defensins; comparative immunology; Histology and Cytology: histophysiology of perinatal and adult gastrointestinal tract and immunocytochemical localisation of growth factors and cytokines; *in situ* hybridisation and Cytogenetics: confocal microscopy for elucidation of chromosomal behaviour and gene activity.

Enrolment

If you wish to enrol for 2000, enrolment packs will be available from the Enrolment Office from October, 1999. They can be obtained by phoning 0800 MASSEY or 0800 627 739. For students who have previously enrolled at Massey University, the deadline is 15 December, 1999. For students new to the university, the deadline is 15 January, 2000.

For further information, contact:
Associate Professor Mary Nulsen, Academic Director, Medical Laboratory Science, College of Sciences, Massey University, Palmerston North. Phone 06 350 4021 Fax 06 350 5609
E-mail M.F.Nulsen@massey.ac.nz

Pigs, Encephalitis and Viruses, A Review.

1999 proved to be a bad year for pigs and those handling them in Malaysia for in that year more than 100 people who worked with pigs died from and of course many more suffered from febrile encephalitic illness. Initially the outbreak was attributed to the virus that causes Japanese encephalitis which is transmitted from pigs to humans by the culex mosquito. It seems that the serological tests carried out early in the outbreak indicated that the illness was Japanese encephalitis but epidemiological evidence suggested the existence of another virus.

Electron microscopy and other tests revealed that the "other" virus was related antigenically to the Australian Hendra virus that caused major illness among horses and their handlers in Queensland. Since the "other" virus was obviously a new virus it was named Nipah virus after the locality of the outbreak. Due to the team work of the Malaysian health authorities, visiting consultants and the CDC, Fort Knox, tracking of the killer virus from isolation to identification took exactly 17 days. Malaysia has been vigorous in dealing with the problem and ordered the culling of pigs and about 1,000,000 pigs have been destroyed. Epidemiological studies were carried out to investigate how the virus was transmitted and if other animals in addition to the pigs were involved. Other carriers of the virus have not been proven and it appears that most resulted from direct contact with pigs. There is no evidence that human to human transmission occurs or that infection can follow the consumption of pork. Laboratory investigations of suspected Nipah virus infections show a raised CSF lymphocytosis and protein and a positive Hendra serology.

Ngerengere in Aotearoa

Bullamakau's recent reading produced references to the following diseases; ngerengere, Elephantiasis grecorum, Lepra gangraenosa, tuwhenua mutumutu and tuhawaiki. It seems that these were early names given to the various forms of leprosy in New Zealand. A number of early medical writers have recorded cases which are most likely to have been leprosy. Indeed the eminent Maori politician Maui Pomare (1904) reported to the House of Representatives: 'There are at present five persons suffering from this (Hansen's) disease in New Zealand and with the exception of one Chinaman, all are Maoris.' Was leprosy here in Aotearoa before the white man arrived? It seems that the names 'tuwhenua' 'tuhawaiki' relate to one of the migration canoes and to the mythical island of origin of the Maori so perhaps the disease was brought to New Zealand from other Pacific Islands by the original inhabitants. It is more than likely though that the disease was introduced by Europeans at the end of the eighteenth century.

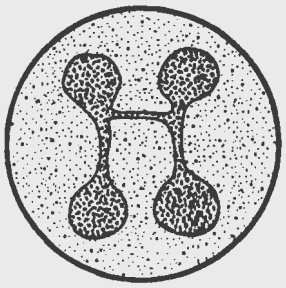
Yaws

The early medical reports on yaws in the Pacific region is most confusing. Cook mistook yaws, which was widespread in the Pacific, for syphilis. By 1840 yaws was highly endemic in Fiji and the study of skeletal remains indicates that yaws had been in the Pacific region and Papua New Guinea for many centuries. Yaws has never been present in New Zealand and even if it had been introduced by the early colonisers the cooler atmosphere which leads to less sweating would have inhibited transmission. It is interesting that in those islands where yaws was common syphilis failed to establish. Did this indicate some cross immunity? Also of interest is the reverse of this picture after the World Health Organization (WHO) carried out a Pacific wide campaign to eradicate yaws from the tropical Pacific islands by mass treatment with penicillin. Following the completion of the treatment there was a noticeable increase in the incidence of syphilis.

Hookworm

Hookworm infection in the Pacific is very common and is the cause of much anaemia and chronic ill health. The earliest reference is 1817 in Tonga. In 1923 it was estimated that over 90% of the Tongan population was infected. A year later a survey in Tonga, Samoa, Tuvalu and Vanuatu noted that *Necator americanus* was nearly universal and had been established a long time. The old world hookworm, *Ankylostoma duodenale* was a more recent introduction from India and China.

The items on leprosy, yaws and hookworm have been abstracted from 'Infectious Diseases: Colonising the Pacific?' by John Miles. University of Otago Press. This small book is of interest to all medical laboratory scientists as well as those non-medical readers who have an interest in the countries and people of the Pacific.



Answers to Journal Based Questionnaire Progress in Understanding the Pathogenesis of the Anaemia of Chronic Disease

1.	T	ACD is defined as the anaemia occurring in chronic infections, inflammatory disorders or neoplastic disorders (where there is not replacement of marrow by tumour cells)
2.	F	It is characterised by an increased serum iron and total iron binding capacity with normal or increased iron stores
3.	T	ACD does not include the anaemias caused by endocrine, renal or hepatic insufficiency.
4.	F	Iron deficiency may be only second to ACD as the most common cause of anaemia
5.	T	There are three pathological processes involved in ACD d) Shortened red cell survival e) Failure of marrow to increase red cell production f) impaired release of iron from the reticuloendothelial system
6.	T	There is an increased response of erythropoietin (EPO) in ACD but this is less than would be expected for the degree of anaemia
7.	T	The failure of the bone marrow to respond to these increases in EPO is the primary cause of anaemia in ACD
8.	T	Tissue necrosis factor (TNF) plays a significant role in inflammation and the immune response.
9.	F	Tissue necrosis factor has been found to be decreased in those conditions causing ACD.
10.	T	TNF appears to inhibit erythroid precursor response but does not affect platelet or granulocytic precursors
11.	F	β interferon (βIFN) inhibits the action of TNF
12.	T	IL-1 has a wide variety of actions in inflammation and immunity and shares many of the properties on TNF
13.	T	IL-1 is elevated in ACD and correlates with the anaemia
14.	T	Recombinant human IL-1 (α and β) inhibit erythroid precursors from normal marrow invitro
15.	T	The inhibitory effect of IL-1α is mediated by TNFα
16.	F	γ interferon (γFN) is produced by B lyphocytes
17.	T	γIFN is involved in the modulation of immune and inflammatory responses as well as the host defence against microbial challenge
18.	T	γ interferon inhibits erythroid progenitors and reticulondothelial iron release
19.	T	The distinctive feature of ACD is a low serum iron in the presence of adequate reticuloendothelial stores
20.	F	The block in iron reutilisation or mobilisation is the cause of the hypoferremia
21.	T	Impairment of erythropoiesis is the most important contributor to the anaemia of ACD
22.	T	EPO treatment has been found to increase the haematocrit of patients with ACD
23.	T	Cytokines inhibit erythroid progenitors, EPO production and impairment of iron release

Journal Based Questionnaire

British Journal of Haematology, Vol 105 (July 1999), 852-863
Myelodysplasia and Myeloproliferative Disorders in Childhood: An Update

1.	MPD and MDS occur in children as they do in adults?	True	False
2.	Childhood MDS occurs with a male predominance of 5:1	True	False
3.	Adult type CMS (i.e. bcr-abl + ve CML) occurs in children	True	False
4.	What is the most common childhood MDS?		
5.	List at least two previous terms for the condition now known as JMML		
6.	The current recommended classification for CMML and JMML is MP/MD disorder?	True	False
7.	List at least three parameters in a Full Blood Count / blood film which may help to distinguish between ACML and JMML in a child		
8.	Interferon is the only potentially curative therapy for children with ACML	True	False
9.	Monosomy 7 is a syndrome which is completelly separate from JMML	True	False
10.	JMML monomuclear cells demonstrate colonly growth spontaneously	True	False
11.	What do JMML haemopoietic cells have selective hypersensitivity to?		
12.	Most patients with JMML have normal cytogenetics	True	False
13.	What percentage will have monosomy 7?		
14.	Degeulated RAS signalling is thought to be the pathogenetic mechanism in the development of JMML in all patients	True	False
15.	What percentage of JMML patients transform to an acute leukaemia?		
16.	What three factors determine prognostic category in JMML?		

For the answers turn to page 123.

For copies of this article please contact Janene Madgwick, email: akspechaem@ahsl.co.nz

Histology

Special Interest Group

Convenor: Ann Thornton
Wellington School of Medicine

The South Pacific Congress which was held in Christchurch from 23 to 27 August, had an excellent programme which catered for all participants in the Medical Laboratory Science field. Of particular interest to histologists was the Histology/Cytology seminar on Thursday 26th. Invited speakers from the USA, Australia, Wellington and Christchurch were included in the programme, with a wide variety of relevant topics. There is no doubt that the day was well planned, and it was a credit to the organisers.

The day began with an enlightening look at proliferation markers in histopathology. Brett Delahunt from the Wellington School of Medicine took us on a brief history of pathology, followed by the evolution of tumour grade and mitotic rate as determinants of prognostic assessment. This led to an explanation of cell cycle activity and recent immunohistochemical markers used to determine stages of the cycle, and therefore useful to indicate proliferation rate. Professor Delahunt explained how the quantitation of these, and the use of a silver staining method for Nucleolar Organiser Regions, can provide significant prognostic information for the clinical pathologist.

Jacqui Gardner's talk entitled "Skin Pathology of the Elderly" showed us many common skin complaints that affect the elderly. Her talk included graphic pictures of lesions in their gross state followed by their histological features. The link between gross and histological features was very beneficial to all.

Nina Fotinatos followed with findings from a study carried out in Victoria assessing types of fixatives including some fixatives which were newly developed. The results of the study were very exciting – it seems we may have the choice of a new, low irritant fixative, with preservative properties similar to formalin in the near future.

Richard Brunning, our international speaker from the University of Minnesota, USA, opened the second session by describing the appearances of malignant lymphomas and related disorders in bone marrow biopsies in the histology laboratory was also discussed in some detail.

The cytology component of the day began with Paul Shield of Brisbane who reviewed the RCPA Quality Assurance Programme in Gynaecological Cytopathology. This programme has been running in New Zealand and Australia for the last six years. Analysis of the slide surveys since 1993 has shown a gradual improvement in performance from the participating laboratories. Paul also revealed new developments to the programme in keeping with new developments in the cytology field.

Di Taylor from Canterbury Health Laboratories gave us a very interesting talk comparing the use of the new Diff-Quik stain to the May-Grunmeld Giemsa. In the talk Di questioned the validity of the two stains and discovered some surprising details.

Chris Bowden provided us with a review of 100 bronchoscopy specimens taken at Christchurch Hospital. He correlated the cytological findings with the histology diagnosis and assessed their value. Collaboration between cytology and histology in these cases was found to be of prime importance.

Anthony Woods is currently using in-situ hybridization techniques in his histology laboratory at the University of South Australia. He gave two talks advocating its use in the histology laboratory and describing probe production techniques. He described the advantages of using in-situ hybridization and explained how readily the procedure can be incorporated into the histology laboratory.

Clyde Riley from Melbourne gave a spectacular slide show of Australian native plants to describe the development of seed extracts for lectin-like activity. He noted that there appeared to be no examples of lectins from Australian species available and recommended the use of a screening process to isolate new lectin-like compounds.

The final talk of the day was given by Jillian Kril from University of Sydney. Jillian provided us with an illustration of the neuropathology that occurs in the alcoholic brain, and the differences that take place in those alcoholics with Wernicke's Encephalopathy (WE) and Korsakoff Psychosis (KP). Thiamin deficiency appears to be important in the aetiology of brain damage in WE, while the alcohol itself appears to be of consequence in KP.

All the sessions were well attended with 30 people at each session. The speakers were of a very high standard and we thank them all for their outstanding contribution.

Finally, a special thanks must go to Stephanie Neal for doing such a wonderful job of organising the Histology session. Without Stephanie's hard work the day would not have been possible.

Answers of the Journal Article
British Journal of Haematology, Vol 105 (July 1999), 852-863
Myelodysplasia and Myeloproliferative Distorders in Childhood:
An Update

1. True
2. False
3. True
4. JMML
5. Childhood CML, JCML, JCGL, subacute MML, chronic MML
6. True
7. Monocyosis, basophilia, platelet count, dysplastic morphology
8. False
9. False
10. True
11. GM-CSF
12. True
13. 25-33%
14. True
15. Approx. 15%
16. Platelet count, fetal haemoglogin level, and age

Transfusion Science

Special Interest Group

Convenors: Geoff Herd, Northland Hospital
☎ 09 438 2079
Sue Baird, Southland Hospital
☎ 03 218 1949

National Immunohaematology Continuing Education Weekend. 1999

The 10th annual National Immunohaematology Continuing Education weekend was held in Wairakei on 14 & 15 May 1999.

A Quality Forum convened by the New Zealand Blood Service preceded the weekend. Topics at the Quality Forum included Management of Risk in Transfusion Medicine, NZBS Quality System Development, Progesa implementation, Update on International Accreditation New Zealand ISO guides for medical laboratories and an open discussion forum.

The NICE activities commenced on Saturday morning with congratulations to Will Perry and Sheryl Khull who have attended all 10 NICE weekends. As is our custom, all participants were required to present a paper on some aspect of the transfusion sciences. Topics included case histories, investigative work, management of transfusion services, blood product forecasting, the effects of shift work to name but a few. The TSSIG would also like to congratulate all participants for their presentations.

This year ABBOTT laboratories sponsored two prizes for the best papers. Helen Norton won the prize for best paper for first time participants for her case study on Paroxysmal Cold Haemoglobinuria. Sheryl Khull won the open section for her paper describing an antibody produced in response to a new variant of the D antigen.

The dinner on Saturday night was complete with party poppers and a huge birthday cake – chocolate of course! Sunday's events were rounded off with a happy birthday serenade to Sheryl Khull who with Christine Van Tilburg have been the convenors of the NICE weekend for the last three years.

On behalf of the TSSIG I would like to express our sincere thanks to Sheryl and treasurer Christine Van Tilburg for their hard work in organizing this very successful meeting. The TSSIG would like to say a big thank you to ABBOTT Diagnostics, Med Tel New Zealand Ltd, CSL Bioplasma, Baxter Healthcare, Dade Behring Diagnostics Ltd, CSL Biosciences Diamed New Zealand, Pharmaco (NZ), Life Technologies Ltd and Mid-Central Health Ltd for your generous support of the NICE weekend's activities.

Report on the South Pacific Congress August 1999.

The TSSIG ran a very successful wet workshop on Aquired Immune Haemolytic Anaemias. All 15 places were filled and the participants had the opportunity to handle real specimens from these patients or see demonstrated the techniques used in the investigation and preparation of compatible blood for these patients.

The TSSIG would like to thank Diane Whitehead, Helen Norton, Adrienne Mackay and Linda Pinder for their generous help and expertise.

TSSIG Activities for next year 2000.

The NICE weekend will be held at Wairakei on the weekend of 28-30 April 2000.

The TSSIG is planning a workshop on Haemolytic Disease of the Newborn and Antenatal Antibody testing to be held in conjunction with the NZIMLS conference in Rotorua.

Eleventh Annual

NICE WEEKEND

A Transfusion Science educational opportunity organised by the TSSIG

at Wairakei on 28-30 April 2000

The NICE Weekend (National Immunohaematology Continuing Education) is an educational meeting for all people working in Immunohaematology and/or blood services . As usual it will be held at the Wairakei Resort Hotel.

As always, all participants are required to participate. You must present either a poster, or an oral presentation lasting 2 to 5 minutes, on any topic related to Immunohaematology or blood transfusion. It can be a case study, a discussion, a question, a problem you want others' help with, etc. This will be followed by questions and discussion of the topic you raise. This compulsory participation makes everyone nervous (yes, even the "old hands") but it really is one of the reasons why the NICE Weekend is so successful.

The registration fee is $250, reduced to $220 for current financial members of the NZIMLS. Your registration fee entitles you to:

- two nights (Friday 28 April and Saturday 29 April) accommodation on a share twin basis
- continental breakfast, morning and afternoon teas, and lunches on Saturday and Sunday
- dinner on Saturday night.

Transport costs will be your own responsibility.
Accommodation on other nights and other meals can usually be arranged directly with Wairakei Resort Hotel. This is also your own responsibility.
Please plan arrive at the venue on Friday evening, as we have a full programme planned.

If this is your first NICE Weekend, we will put you in contact with a "buddy" who can introduce you to everyone, explain anything you don't understand and make you feel at home.

Because **participant numbers are limited to the FIFTY registrations preference will be given to NZIMLS members .** We will fax your application form back to you on receipt, to let you know that your registration has been received . If you don't hear from us we have not heard from you.

If you have any questions contact

Raewyn Cameron *or* *Andrew Mills*
ph 07 349 7908 *ph 07 839 3679*
email cameronr@lhl.co.nz *email*
andrew.mills@nzblood.co.nz

NICE WEEKEND
28-30 APRIL 2000
A Transfusion Science Education Opportunity
Organised by The TSSIG

Please register me for the 2000 NICE Weekend

Surname:		**First Name:**
Address for receipt:		
Phone:	**Fax:**	**E-mail**

Paper or Poster: (Circle) Title:

A brief abstract of your presentation MUST accompany your registration form by 25th March 2000

Is this your first N.I.C.E Weekend? Yes/No

Registration Fee **Or for NZIMLS members**	**- $ 250** **- $ 220**	**$**
Private Room Surcharge **I wish to share a room with** **.....**	**- $ 125**	**$** _____
Late Registration Fee (payable after 25th March)	**- $ 50**	**$**
I enclose a cheque, made out to "NICE WEEKEND" for the amount of:		**$**

Applications received after Friday 25th March 2000 can only be accepted if accompanied by the late registration fee. The Private Room Surcharge is payable only if you wish to have a room to yourself.

Signature:

Please send form and cheque to Andrew Mills, New Zealand Blood Service – Waikato, P.O. Box 185, Hamilton. Note registration is limited to 50 participants and preference will be given to NZIMLS members .

Your form will be faxed back to you promptly to confirm your accepted registration.

Registration received and accepted	**Date:**

New Products and Services

New Products from CLS

AUTOZYME™ ANCA

Designed with flexibility in mind, Cambridge Life Sciences is now offering either qualitative or quantitative detection of antibodies to both PR3 and MPO in human serum. The new AUTOZYME™ ANCA ELISA can be used to greatly aid the differential diagnosis of Wegener's Granulomatosis and other autoimmune vasculitides. Both kits utilise high purity native antigens with guaranteed excellent specificity. A simple method with 30 minute room temperature incubations, together with the added convenience of common reagents and methodology, ensures that the AUTOZYME system is well adapted to automated technology.

AUTOZYME™ GBM Anti-collagen IV α3

Cambridge Life Sciences has launched AUTOZYME™ GBM, an ELISA for the measurement of anti-collagen IV α3 antibodies. Good Pasture's Syndrome (GS) is an autoantibody mediated glomerular nephritis characterised by the presence of antibodies to the α3 chain of type IV collagen which is the specific antigenic determinant for the glomerular basement membrane. Early detection is vital for the successful treatment of patients with GS. AUTOZYME™ GBM utilises ready-to-use, colour coded reagents and has dual protocol to give either qualitative or quantitive results in 90 minutes.

For more information, please contact us.
Med-bio Limited
ph: 0800 MEDBIO
fax: 0800 101 441
email: jvincent@medbio.co.nz

Important announcement:

Hettich centrifuges will now be distributed in New Zealand by med-bio limited.

Hettich centrifuges feature the latest technology in new design along with established quality. From bench top centrifuges to refrigerated centrifuges. There is a Hettich centrifuge which is right for your laboratory's requirements.

For more information about these exciting products, please contact:
Med-bio Limited
Rose-Marie Daniel North Island
Julie Vincent – South Island
ph: 0800 MEDBIO (633 246)
fax: 0800 101 441
email: jvincent@medbio.co.nz

Reliability of tests upheld

We have heard a great deal in the media lately, about cervical cancer testing going wrong, about DNA testing giving incorrect results, about blood alcohol meters incorrectly calibrated and equine (horse) blood samples wrongly labelled.

So how can people rely on the experts to detect disease from their blood/biopsy samples?

How do we know if the reading on the alcohol-testing device, or speed camera is right? Or that the DNA presented as evidence in a court of law has been accurately identified? Are goods that carry claims that they have been tested safe? Was the right test performed, and was the testing laboratory sufficiently competent to perform the test?

Against the very serious events of recent months, there are some real assurances. People can have real confidence in their test results from a laboratory accredited by International Accreditation New Zealand (IANZ). In New Zealand, accreditation of a laboratory means a great deal. Laboratories are accredited against the International Standards Organisation's (ISO) stringent guidelines. Not only are accredited laboratories required to rigorously follow testing and checking processes, but the people who perform the tests also have to meet strict standards of competence.

In the above four cases, none of the laboratories were accredited against the ISO requirements. Accredited laboratories are not immune from errors, but have systems to detect and rectify human error. Users of accredited laboratories will have greater confidence that errors such as those highlighted in recent months will be corrected before results are released. Importantly, accredited laboratories can trace back the circumstances surrounding any incorrect result.

In the last decade, the quality assurance process has grown much tighter in ensuring that the testing method is technically valid and the process is quality controlled right the way through.

IANZ is a statutory body whose main business activity is the accreditation of testing laboratories. Although IANZ accreditation of laboratories is not compulsory, public pressure, the regulatory requirements of organisations like Ministry of Health and MAF, and professional responsibility have motivated all but a handful of laboratories in New Zealand to become accredited. These 800 accredited laboratory units conduct literally millions of tests every year. And of those, only a tiny number are found to be inaccurate.

As well as having one of the highest number of accredited laboratories per head of population, New Zealand has also taken a lead in becoming the only country in the world to accredit radiology services, and to accredit medical laboratories to the full ISO Laboratory Accreditation standard.

IANZ accreditation requires laboratories to participate in inter-laboratory comparison programmes in which samples are swapped, and results and diagnoses compared. Even when someone is working in isolation, there are now mechanisms in place to pick up errors. The stringent guidelines include internal audits and continuous qulaity checks with every batch of tests. Accredited laboratories must have external checks, and systems to ensure that test results are reliable. The guiding principle with accredited laboratories is that their test results should be the same as those of other similar laboratories if they tested the same samples.

Commercial imperatives as well as public concern for the safety of goods, and the validity of tests have driven the growth of internationally recognised standards to which industries must now conform. The scope of IANZ's accreditation coverage includes inspection and testing ranging from testing the safety of electrical devices and gas cylinders to building materials, road construction and food. Environmental testing is another important area.

Most countries now use the ISO standards as a basis for conformity assessment. This has huge implications for trade. To ensure safety, protection against fraud, and to minimise environmental effects, our trading partners are increasingly relying on accredited test results. And this reliance will grow as food products also come into the free trade zone.

Regardless of whether the issue of accurately tested and labelled products concerns trading partners or individuals, the interested parties should be checking every time to ensure that the test results on which they rely have been perfomed by a laboratory with accreditation from an internationally recognised body such as IANZ.

For more information, please contact:
Dr Llewellyn Richards
Chief Executive
International Accreditation New Zealand
Tel: 09 525 6655
Fax: 09 525 2266
Email: lrichards@ianz.govt.nz

Abstracts, South Pacific Congress, Christchurch

Social aspects of living with HIV. The current situation

Mr Kevin Hague, Executive Director, Aids Foundations, Auckland

As the number of people living with HIV in New Zealand continues to climb steadily, we are effectively a nation in denial, with large sectors of the population believing the epidemic to be over because it hasn't yet touched them, and the widespread use of ineffective strategies to prevent further transmission. Recent events have raised the real prospect that almost nothing has changed over the past 15 years in the way that our society views HIV, with catastrophic consequences for people living with HIV. This paper reviews New Zealand's management of the epidemic.

Update on HIV in the USA/Laboratory tests available to monitor HIV infection, including opportunistic infections.

Dr Larry Reimer, Chief Infectious Diseases and Clinical Microbiology
VA Medical Centre, Salt Lake City, USA

HIV in Utah is a common but not overwhelming problem with the state ranked about 25th of the 50 United States. The demographics of the infection in this state will be presented along with medical approaches to care including the drug regimens commonly employed. The approach to laboratory testing to monitor the stage of infection and the effectiveness of therapy will be briefly discussed. New protocols for HIV drug resistance testing will also be mentioned.

Utah also has an extensive network of support services including housing assistance, a food bank and food delivery program, homemaker services, home health aides, limited financial assistance, and psychological and psychiatric support services. These will also be described. Time will be allowed for questions from the audience.

Development — Vaccines and new Treatments and Prevention of HIV

Charles F. Farthing, MD
Medical Director, AIDS Healthcare Foundation
Assistant Clinical Professor of Medicine, UCLA

Over the last three years, spectacular changes have occurred in our ability to manage HIV infection/AIDS. Now triple antiviral therapy (with or without protease inhibitors) is controlling HIV infection in most patients in the developed world and immune systems are reconstituting. Death rates have fallen dramatically. The challenge for HIV clinicians is now not so much finding an effective therapy, but keeping people on it; most deaths are now occurring in individuals who are non-adherent to their therapies. Many challenges still exist however we need therapies that are easier to take with fewer side effects and that will treat drug resistant HIV. Many such therapies are currently in development.

The quest for the microbiological "cure" for AIDS (the eradication of the viral genome from the body) goes on but prospects seem dim. A glimmer of hope exists however that some individuals may ultimately be able to come off therapy with long term immune control of a small residual noneradicated population of HIV infected cells. Ultimately worldwide control of this disease however, will only be obtained by development of an effective, safe HIV vaccine. Dead vaccines have so far been singularly unsuccessful in protecting uninfected individuals. Live vaccines, although effective, are considered too dangerous to develop as a certain percentage of vaccinated individuals may progress to disease. Research continues with perhaps the most promising vaccines being those utilising naked DNA or live vectors.

Recent develops in treatment and vaccine development will be reviewed in this presentation.

Integrate Care Project for the Elderly

Elder Care Group, Christchurch

The Elder Care Canterbury Project is a project that will deliver ongoing incremental improvement and innovation in the delivery of a comprehensive patientfocused health service for older people in Canterbury.

What are the key objectives?

The key objectives for the project are as follows:

- To develop a comprehensive health service for older people in Canterbury
- To work with the community in an inclusive and collaborative way, to develop the best possible service.

- To focus the skills, knowledge, enthusiasm and commitment of the existing local providers and the wider community to design a service that meets the specific needs of the older people of Canterbury.

What is the target population?

The focus is on people in Canterbury over the age of 75 years but including those younger who have health disorders associated with or complicated by the aging process. Canterbury is defined as the area covered from Kaikoura to Rakaia and east of the Southern Alps. Christchurch with a population of 360,000 is the metropolitan centre for the area.

The demographic details are as follows:	1996	2021
65 and over	51,130	85,270
75 and over	21,550	36,120
85 and over	4,710	10,910

Who is involved?

- Patients, their Family and Carers
  The project is focused on the provision of services that meet the needs of patients, their family and carers. To ensure that this focus is maintained patients, their family and carers are actively involved in the process of developing new models for health service delivery.
- Providers
  The initiators of the project were Pegasus Medical Group GP's and Healthlink South Geriatricians. Canterbury Health and Healthlink South joined at an early stage. Since that time the project has expanded and now includes all providers (Primary Care urban and rural, Secondary Care, Residential and Community Care).
- Stakeholders
  The Community stakeholder group, which meets monthly, has representatives from a range of community groups including, Age Concern and Greypower.

The Clinical and Disability Support Provider group, which also meets monthly, has representatives from community and residential care providers.

The Crown is represented in the project through ACC, Crown Public Health, the Health Funding Authority and the Ministry of Health. Each of these organisations is kept fully informed of progress with the project and representatives from these organisations are actively involved in individual projects. The Minister of Health, Shadow Minister and all local politicians are also kept fully informed.

The Health Service Provider group represents all providers in Primary and Secondary Care.

How is integration to be achieved?

Integration in the context of this project is about changing minds and attitudes to enable the provision of a seamless health service for older people.

Integration is to be achieved through a series of individual projects. Each individual project will develop and then implement a new model for a service for a specific disease entity.

What are the individual projects?

The initial projects are as follows:

• Acute Confusion • Broken Hip • Stroke

In addition a generic project on Positive Aging is addressing the need for prevention and wellness strategies to improve the health of older people. Other projects will be developed once the initial projects have moved to the implementation phase.

How are the projects developed?

Project team leaders are identified for each project. The team leaders work with the Steering Group and project coordinators to confirm the scope and time lines for each individual project.

Project teams develop the individual projects. The project teams include representatives of the community as well as Primary Care, Secondary Care, Residential and Community care. Project team members are selected for their experience knowledge and expertise in their particular field not on the basis of seniority or title.

Is there a methodology for the project?

A standard methodology has been developed for the project and is applied to each individual project. The methodology is as follows:

- Establish a project team.
- Identify the current service and document.
- Circulate document to Stakeholders for feedback.
- Develop new model for service and document.
- Circulate document to Stakeholder for feedback.
- Implement new service.

What is the structure of the project?

All individuals or groups with an interest in the provision of services for older people must be included in the project. To achieve this the project remains open to membership from any individual or group. There is therefore no formal structure for this project. Such a structure would imply the inclusion of some individuals, professional groups, organisations, and institutions and the exclusion of others.

There is a Memorandum of Understanding between the three organisations that initiated the project (Canterbury Health, Healthlink South and Pegasus Medical Group), This Memorandum addressed administrative matters.

How is the project managed?

The management of the project is achieved through the following:

- Executive There are four people on the Executive. These include the CEO's of Canterbury Health and Healthlink South, and the Chair of the Pegasus Medical Group. The Executive has overall responsibility for the project.
- Steering Group There are eight people in the Steering Group, two from each of the following:
  Canterbury Health, Healthlink South, Primary Care and Residential and Community Care. The Steering Group is responsible for setting the objectives and time lines for the overall project and for the individual projects.
- Project Facilitator The project facilitator is independent of any of the organisations involved in the project. This independence is critical to the success of the project. The project facilitator is responsible for the leadership of the project, chairs the Steering Group and leads the Project Coordinators.
- Project Coordinators There are four Project Coordinators. These people are employed in each of the following Canterbury Health, Healthlink South, Primary Care and Residential and Community Care. They are seconded by their organisation to the project for up to 10 hours per week. The Project Coordinators are responsible for ensuring that the objectives and the time lines set for the individual projects are achieved.
- Project Administrator There is a full time project administrator who provides a full administrative and secretarial service to the project.

What are the time lines?
The project commenced in July 1997 with the first meeting of the initial partners.

From July until December 1997 a project team with 4 representatives from each of these partners met on a weekly basis to develop a model for the project. In January 1998 a Steering Group was established.

Stakeholder groups were established in February/March 1998.

Project teams for the initial projects were established in March/April 1998. The first project team report (Broken Hip) was presented in June 1998.

This is an ongoing project that is continually expanding as all providers begin to work together in a collaborative and inclusive manner to deliver a comprehensive and patient focussed service health service for older people.

What was achieved in 1998?
- Development of relationships between Primary, Secondary, Residential and Community care providers.
- Development of relationships with Stakeholders (Community, Clinical and Disability Support Providers, Health Service Providers GP Focus group)
- Changing of focus both individually and collectively from a professional and institutional bias to a common vision for patient focused services.
- Development of a model for a broken hip service.
- A combined project to maximise the impact of the Influenza Vaccination Program for the over 65's which achieved in excess of 70% coverage.

What are the objectives for 1999?
The specific objectives for 1999 are as follows:
- To continue to develop a common vision for patient focused services.
- To develop and implement the new services proposed by the Acute Confusion, Broken Hip and Stroke project teams.
- To continue to develop meaningful relationship with all Stakeholders.

What is the significance of this protect?
This is the only integration project in New Zealand addressing the issue of health services for older people. The project has attracted significant interest from a wide crosssection of people throughout New Zealand. The project has also raised International interest.

The Elder Care Canterbury Project is one of the Health Funding Authority's National Demonstration Integration Projects.

How is information about the project communicated?
Communication is critical to the success of the project.

Project updates, which give a current review of progress with individual projects, are circulated to in excess of 1600 individuals and organisations at the beginning of every month. Project reports are circulated to all interested individuals and groups.

The project has a web page (http://www.ecc.net.nz). All project reports are posted on this page. In addition project team membership, meeting minutes and other relevant information are accessible through the page.

Is information about the project readily accessible?
The focus of the project is on total transparency.

Any individual interested in the project may attend any of the meetings. All of the information (agenda, minutes, reports etc) about the project is also available to any individual or organisation.

Contact Information

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Factors influencing brain pathology in the elderly

J.J. Kril, K. Double, A.J. Harding* and G.M. Halliday**
Centre for Education and Research on Ageing, The University of Sydney,
*Sydney and *Prince of Wales Medical Research Institute, Randwick, Australia*

Impairment of brain function in elderly may be as a result of an age-related decline in the brain itself or due to the presence of a neurodegenerative disease. We examined the hypothesis that brain atrophy and neuronal loss are a normal consequence of ageing by studying a population of normal elderly subjects without evidence of cognitive impairment and comparing our findings with similar measures from patients with Alzheimer's disease. The volume of the grey matter and white matter components of each lobe of the brain was measured using a point counting technique. The number of neurons in the CA1 region of the hippocampal formation was also determined in normal aged subjects and patients with Alzheimer's disease. During ageing no decline in grey matter volume is observed, however there is a small (~2mL/year) decline in white matter volume. In addition, no loss of neurons from the hippocampus is seen. In contrast, patients with Alzheimer's disease have significant atrophy of the frontal and temporal lobes and a marked loss of hippocampal neurons. These findings indicate that significant brain atrophy and neuronal loss are not experienced during ageing.

It's a Journey, Not a Destination!: How the Australian Red Cross Blood Service is Travelling

Dr Patrick Coghlan, Australian Red Cross Blood Service Victoria, Melbourne

In November 1995 the Australian Red Cross Society created the ARCBS, following the acceptance of the recommendations of the McKay-Wells report, which was an inquiry into the Australian blood system on behalf of the Commonwealth government. McKay-Wells raised seven key issues that became the focus of action for ARCBS.

The first issue related to the complexity of structure and systems that prevailed in the separate ARC blood services of the day including weak national authority, and to the growth in annual expenditure. The second issue related to sufficiency of blood products. Thirdly, the separate services were regarded as demonstrating too much variation in structure, practices and managerial expertise.

Issues four and five highlighted the deficiencies of extant risk management systems and lack of standardisation of human resource policies and practices. The sixth and seventh issues dealt with the lack of coordination of R&D and lack of ability in developing new business opportunities.

These seven issues will be used as a framework for examining where ARCBS is, in relation to the programs of work that were created as a means to achieve the objectives set by the Board and Executive. The heroic scale of this change program will be emphasised.

One year into the NZBS from a Medical Perspective

Dr Peter Flanagan, Medical Director, NZ Blood Service, Auckland

NZBS was established with responsibility for the development of an integrated national approach for delivery of transfusion services within New Zealand. A number of basic principles can be identified to support this development. These include a commitment to consistent access to services and products, consistent standards for both the manufacturing and clinical aspects of transfusion medicine, and development of systems to support these goals.

Frameworks have been put in place to support key strategic goals. Two key advisory groups have been established. A Clinical Advisory Group, this includes all Transfusion Medicine Specialists within New Zealand and also the National Quality Manager. A Blood Services Advisory Group's membership includes a combination of professional and consumer representation.

NZBS is committed to providing services and products that meet international standards in the transfusion field. Observer status has been achieved on the Council of Europe (CoE) Committee responsible for Guidelines and Standards. The CoE Guide on the manufacture of blood components will provide an external focus for revision of the New Zealand Standards. The NZBS minimum standards will be updated to define specific standards and requirements, which will be applied consistently at all sites across the country.

Quality Development leaders have been appointed at all future major processing sites, together with the National quality manager these will support implementation a national quality system. This is seen as a high priority for the service.

Significant progress has been achieved during year one. In particular the foundations have been established to ensure that the strategic agenda identified by NZBS in 'Looking Forward — The Vision for a World Class Blood Service' will be fulfilled.

One Year into the NZBS from an Operational Perspective

Jennifer Mitchell, General Manager Operations NZBS, Auckland

The first year for NZBS has been an establishment year. Goals and objectives have been set for the development of a national service.

Three major projects are now well underway to achieve these objectives:

The Blood Management System which implements a national Information Management System.

The Integration Plan which involves integrating blood services from twenty-one other Hospital and Health Services

The Service Development Plan, which outlines changes to the way services will be delivered, in particular the centralisation of process and accreditation.

Along with these projects the infrastructure, policies and procedures of NZBS have been developed to support the day to day operations as well as major and minor projects. Systems for Finance, Contracts and Human Resources together with principles for decision making have been created within the new company. NZBS is moving from a setup phase to that of growth and development.

Space developments and Earth applications

Dr Vaughan Clift, Physician, NASA, USA

Most people are aware of the dramatic achievements of the space program and some of the technological advancements that have been stimulated by the race to the stars. However, most are unaware of the tremendous amount of ground based research that is required simply to make an experiment or observation possible in space.

Since the long duration flights of Skylab a great deal has been learned about how human's respond to spaceflight. In fact, more has been brought into question that probably resolved in any referencable manner from this data. The shear complexity of flying even the simplest experiments and the small numbers of subjects available per flight or per annum, have made the process a long and arduous one.

However, the little that has been gained has often brought into question accepted understanding of human physiology. The fact that osteoporosis, accelerated by microgravity, is reversible in postmenopausal astronauts and that red cells congest the marrow during flight provide potential insights into important diseases on Earth.

The vast majority of laboratory equipment that would be used to study these changes on the ground do not function in microgravity. This has forced the scientist to invent new ways to collect samples and perform assays. These new methods are proving to have a greater benefit on Earth.

Cannabis, All You Need to Know!

Sharon Paterson, Toxicology, Canterbury Health Laboratories

From the 28th century BC, cannabis has played a major role in medicine and religious ceremony. Its spread into the West only occurring last century, with the social problems and consequences of its abuse really only hitting us in the last 30 - 40 years.

The side effects range from euphoria to apathy to impotence and paranoia.

Its detection time in urine can be weeks, and as a result, interpretation of levels can be a nightmare for both the laboratory and the clinician. Then there are the legal consequences, not to mention 'new use' versus 'residual excretion'.

An overview of the detection techniques will be presented along with the latest theories on result interpretation.

Concepts of Workstation Consolidation and Integration

Lance Little, Product Manager, Roche Diagnostics

The pressures that face laboratories in today's medical environment are becoming more and more focused around efficiencies, both in terms of result turnaround time and costs. There are many areas within a laboratory where these efficiency gains can be made, and many laboratories have identified the fact that the more analysers there are on the bench, the more it is going to cost. Workstation Consolidation is the term applied to the process of placing as many assays as is appropriate onto one platform, therefore directly reducing the costs associated with service contracts, staff time required for daily setup and training. Improving the turnaround time for a wider range of tests can make added gains; eg. those that would normally have to be batched can be run 'real-time' therefore improving service and reducing the likelihood

of an urgent request being made at a later date.

The second aspect that a few laboratories are looking into now is the cost of the Pre Analytical phase of testing. When we look at both these aspects in combination it is plain to see that laboratories in the past have been organised around like technologies, but in order to maximise any efficiency gains perhaps laboratories should be organised around like processes, or processes that involve the same sample type. Therefore should we be looking at the chemistry, protein, and immunoassay areas within a laboratory as a 'Serum Workarea' whereby the results are generated on high volume analysers? Where necessary the results are interpreted by specialised personnel, who are employed for their knowledge rather than their ability to place samples on an analyser.

Laboratory Automation: A Stepwise Approach to "Flexible Automation"

Dave Daly, Abbott Diagnostics, Chicago

Laboratory Automation - What's the right choice for your lab? With so many options being offered by diagnostic vendors and automation manufacturers, it can be difficult to determine what the best solution might be for each lab's unique requirements. To assist in this process, laboratories should remember to ensure that their automation decisions allow for true flexibility. Flexible Automation decisions can provide labs with the opportunity to take advantage of today's rapidly changing healthcare environment. However, before making any decisions, laboratories must first have an overall automation strategy in place.

As an alternative strategy for laboratory automation, the Stepwise Approach is gaining momentum. This approach involves the evaluation of process improvements, data management enhancements, and finally lab automation components (front-end, workcells, back-end, etc.). The focus of this strategy is to explore and implement lab automation in a step by step process. Potential automation customers need to first carefully evaluate laboratory processes and the opportunity for improvements (workflow analysis, computer simulation, reengineering). Second, data management enhancements should be considered. Well thought-out data management systems can greatly enhance the capabilities of any overall automation decisions. Finally, only when process improvements have been identified and implemented, and the optimal data management system is in place, should the laboratory explore the options that exist for lab automation.

Market data reveals that as much as 65% of a laboratories hands-on time is associated with pre-analytical activities, such as sample sorting, centrifugation, decapping, aliquotting, and racking of tubes into instrument specific racks. This represents by far the greatest amount of labour time within the lab. As a result, to maximize productivity gains while watching costs, front-end automation alternatives are a natural first step in the overall automation process. The next largest area for hands-on time in the lab is in the analytical phase. Diagnostic vendors are addressing this need via Workcells. When front-end automation systems and analytical workcells are combined, data shows that 80% of the hands-on labour within the lab can be impacted. By automating through the combination of pre-analytical workcells and analytical workcells, laboratories can maintain maximum flexibility, while taking a Stepwise Approach to ensure the right level of automation with the maximum return on invested dollars.

Endocrinology of Ageing Youthful Hormones

Dr Chris Florkowski, Clinical Research Physician, Department of Endocrinology
Christchurch Hospital, Christchurch

The ageing process involves alterations in body composition and a decline in physiological functions that are postulated to be related to changes in endocrine systems. An increased understanding of these mechanisms has led to attempts to delay the ageing process through hormone "replacement" strategies.

Gonadal steroids decline during ageing with a fall in oestrogen ("menopause") and testosterone ("andropause"). There is also a decline in adrenal dehydroepiandrosterone and its sulphate (DHEA and DHEAS)("adrenopause") and in the Growth Hormone / IGF1 axis ("somatopause").

Oestrogen replacement is well established for the treatment of climacteric symptoms, the prevention of osteoporosis and possibly atheroscierosis.

A few studies on testosterone replacement in the frail elderly have shown positive effects on muscle strength and well being, though with potential unfavourable effects on haematocrit, lipids and prostate.

DHEA reaches a serum concentration 10 times that of any other steroid hormone in young men, but falls to 20% of its peak value between the ages of 35 and 70. Its wide availability in certain countries coupled with oversimplification of key issues by the mass media has led to its uncritical acceptance as an "elixir of youth". A few randomized controlled trials of DHEA (mostly in nonelderly subjects) have shown an increase in IGF1 bioavailability, physical and psychological well being. There is limited evidence for enhanced immune function, yet concerns exist over the safety of associated changes in other steroids.

The application of agespecific reference ranges for IGF1 has enabled the distinction to be made between the agerelated fall in Growth Hormone (GH) secretion (approximately 10% per decade) and true deficiency associated with hypothalamic pituitary disease (where GH therapy is licensed). GH administration to "normal" elderly men has been shown in some studies to result in favourable changes in bone mass and body composition. Orally active GH secretagogues that act at pituitary level have also been shown to restore IGF1 levels in the elderly to those encountered in young adults.

It is anticipated that the application of anabolic hormones to delay the ageing process will accelerate in the 21st century. The strategy of increasing blood hormone levels in ageing individuals to those found in midadult life, however needs to be critically evaluated.

Can Vitreous Humour collected Post Mortem be used to diagnose a recent Myocardial infarction?

Andrew Humphrey, Department of Clinical Chemistry, Auckland Hospital

Background

Vitreous Humour collected post mortem can be used for the measurement of electrolytes, creatinine and drugs, and can help in establishing the cause of death.

Aim

To establish whether the cardiac enzymes CK and AST, and the new cardiac markers Troponin T and I, could be measured in vitreous humour and whether they might be of some diagnostic utility.

Method

Vitreous Humour was collected from either left or right eye during 22 routine post mortems. The samples were centrifuged at 3000 rpm for

ten minutes and analysed using an Hitachi 911, an Elecsys 2010 (Troponin T) and Axsym (Troponin I). Details of the cause of death, and of the heart histology were recorded.

Results

The two cardiac marker proteins Troponin T and I were not detected in any of the vitreous specimens. AST and CK were found in variable amounts although there was no correlation between the levels of the two enzymes. There was also no relation between vitreous AST or CK levels and whether the patient had had a recent myocardial infarction or not. CK isoenzyme analysis showed prominent CK-BB in selected samples.

Conclusions

Even though CK and AST can be measured in vitreous humour, they were of no value in detecting recent myocardial infarction. False negative results could have occurred because of delayed release of these proteins from heart muscle false positives, because of enzyme release from non-cardiac muscle or the brain. Troponin T and I were not detectable in vitreous humour.

Newborn Screening for Congenital Hypothyroidism - replacement of in-house TSH Radioimmunoassay with Delfia assay

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National Testing Centre, Auckland Healthcare

Newborn screening for congenital hypothyroidism (CH) using an in-house radioimmunoassay (RIA) has been part of NZ healthcare since 1977. Early treatment facilitated by screening prevents the mental retardation associated with this condition but the benefit is compromised if test results are delayed. It was decided to change to a commercial assay since difficulties were experienced obtaining supplies of reagents. The Delfia technology was chosen because it is also available for the other screening assays (17-OHP, Immunoreactive trypsin), fits with strategic plans for future automation and cost.

Comparison of the two assays using patient samples and control material from CDC (Atlanta, GA) showed no bias between them (R=0.999). Other assay parameters are given in the table.

	RIA	Delfia
Interbatch Precision, %	27	11
Intrabatch Precision, %	14	7
Staff time, hrs / day	4.2	1.8
Days to produce confirmed result	4.8	1.7

The Delfia assay is more expensive (reagents plus staff time plus equipment depreciation) but is better for Health and Safety (no radioisotopes). The cut-off has been reduced from 18 to 15 mIU/L blood without increasing the recall rate (potentially detecting more mild CH), and reduced the average age at which likely CH is notified. The benefits (improvements in screening parameters, health and safety) amply compensate for the increased assay cost.

Bone Markers in Osteoporosis

Dr Nigel L Gilchrist, FARCP, Consultant, Princess Margaret Hospital, Christchurch

The development of specific Biochemical Bone Markers has proved to be a very useful research tool, but questions are being asked as to whether they are of use in clinical medicine. The markers of bone formation are serum osteocalcin, alkaline phosphatase and recently bone specific alkaline phosphatase. Markers of bone reabsorption include, urinary calcium, hydroxyproline and pryridinoline, more sophisticated assays involve Deoxpyridinoline, N-Telopeptide and C-Telopeptide. Further markers are also being developed. The difficulties that arise with these assays include geographic and ethnic differences, circadian rhythms and assay variation. There appears to also be confounding effects with a large biological variability and seasonal variation. The intra-individual variation of these tests can vary from 15-50%. The intra-individual coefficient variation of these bone markers over a 24 and 30 month period can be anywhere from 10 - 47%. This large variation in the urine markers is somewhat improved by markers in the serum with values of between 5-16%. Some studies have shown that the use of these urine and serum and markers of bone turnover may predict the response the bone will have to an anti-reabsorption agent such as Alendronate. That is that changes in reduction in osteocalcin and telopeptide predict the potential increase in bone density. However, longer-term studies over four years have shown very poor correlation. Recent work with a selected estragon receptor modulator does indicate some predicability in decrease in bone markers and subsequent increase in bone density. It is hoped in the future with the development of more specific resorption markers that could be measured in the serum, that these will enable us to have more precise measurement. Currently in clinical practice, alkaline phosphatase and bone specific alkaline phosphatase is used to manage Paget's disease. Urinary and plasma telopeptide may enable short term prediction of whether medication given for Osteoporosis is being effective in decreasing bone turnover and perhaps high levels of bone markers coupled with low bone mineral density may help therapeutic decisions.

Nutritional Status of the Elderly

Dr Tim Wilkinson, Princess Margaret Hospital

Undernutrition in older people has a prevalence of 15-60% depending on definition and context. The most important form is protein / energy undernutrition (PEU) although deficits in vitamins and minerals are separate considerations.

Low body mass index is one marker of PEU. In contrast to in younger people, BMI has a U shaped association with mortality. PEU is also associated with an increased rate of post operative complications, higher prevalence of pressure sores and with impaired immunity. Other markers of PEU are plasma albumin, pre-albumin, cholesterol, arm muscle area, skinfold thickness and lymphocyte count. Unfortunately these are non-specific and may be confounded by other disease states. The correlation with poor outcome may also be confounded by comorbidity or social circumstances. There has however been some intervention studies showing a benefit of high protein and high-energy supplements.

Vitamin D deficiency is common in less mobile or institutionalised older people and is associated with low bone density. Vitamin D and calcium supplementation results in fewer fractures in institutionalised older people.

Low thiamine concentrations are more common in older people compared with younger people but there is little evidence that this is associated with adverse sequelae or with comorbidity. Supplementation may offer a small benefit.

There is a very limited place for vitamin supplementation in otherwise healthy older people. Some vitamin supplements may cause harm. High protein and energy foods are likely to provide benefits to

some hospitalised older people. There are well-described advantages of a varied diet high in fibre and low in salt and refined sugars.

An Overview of the proposed World Health Organisation Classification of Leukaemias and Lymphomas

Dr Richard Brunning, Department Of Laboratory Medicine and Pathology
Division of Haematopathology, University Of Minnesota, USA

Previously, the recognition and classifications of leukaemias and lymphomas have been made on cytological and histological; appearances with a few cytochemical stains. In 1976, acute myeloid and lymphoid leukaemias were defined by the French American and British (FAB) Group in 1976, and updated in 1985.

Since the 1960s several attempts to classify lymphomas have been made by a number of different groups, and include the Keil, Rappaport Classifications, and the Working Formulation.

The Revised European and American Lymphoma (REAL) Classification (published in 1994) was devised to include biological information derived from the new techniques of phenotyping, genotyping and cytogenetic analyses, and clinical studies.

In 1996, The World Health Organisation sought to broaden the consensus findings of the REAL Classification, and to include updated classifications of myeloid and lymphoid leukaemias. Committees of European, British and American haematopathologists and clinicians were set up to achieve this objective and the proposed WHO Classification of Neoplastic Diseases of Haemopoietic and Lymphoid Tissues was introduced by the European School of Haematology in London April 1998.

A description of this new classification will be given with particular reference to the use of the laboratory techniques of phenotyping, genotyping, and cytogenetic analyses.

The Human Geneome Project And Beyond — Implications For Health And Health Care

Dr Michael Sullivan FRACP PhD, Paediatric Oncologist and Cancer Geneticist Cancer Genetic Laboratory, Department of Paediatrics and Biochemistry
Medical School, University of Otago, Dunedin

When the Human Genome Project (HUGO) was first mooted in the late 1980s, its goal of mapping and sequencing the human genome seemed extraordinarily ambitious and a long way into the future. However the remarkable pace of molecular technology has meant that the initial objectives of the project have all been met within the planned time frame. It is now apparent that the original 15-year project period was realistic. In the next 3-5 years we can expect that most of the 3 billion bases of the human genome will indeed be sequenced and all the known will have been identified.

So what does the Human Genome Project mean for medicine and health care in the long term. It is clear that disrupted genetics is central to the pathogenesis of many of our major disease such as cancer, cardiovascular disease and CNS disorders — either as inherited mutations or acquired disordered gene expression. The Human genome project has already resulted in the identification of several hundred disease causing genes and over 10 000 genetic syndromes have been identified.

We are now at an extraordinary turning point in genetic medicine with the development of Gene Chip technology. DNA chips will allow us to study thousands of genes from any tissue or patient in a single experiment.

The implications of these new technologies, benefits and risks, for medicine and health care will be discussed.

The Role of Immunophenotyping in the Diagnosis of Granulocytic Sarcoma

J. Monteath, J. Evans, J.Cochrane, I. Llewellyn, M. Brozovic and N. Patton
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Granulocytic Sarcoma (GS) is a rare extramedullary tumour consisting of immature myeloid cells. Males and females are equally affected usually in the third and fourth decade.

Half of the cases occur as blast crises in the course of a known myeloproliferative syndrome including Chronic Myeloid Leukaemia, Polycythaemia Rubra Vera and Hypereosinophilic Syndrome. Approximately 20% have Acute Myeloid Leukaemia (AML) at the time of diagnosis of GS. 30% of patients have no underlying disease at the time of diagnosis and most of these will progress to AML (with a mean survival of 10 months).

GS is often misdiagnosed as Large Cell Non-Hodgkin's Lymphoma. It may occur in the subperiostial region of the bone and is found in sites such as the skull and long bones, the skin, lymph nodes, intestine, sinuses, meninges and breast.

Two case reports of GS are presented where conventional histological evaluation is complemented by MGG stained imprints, immunophenotyping by flow cytometry, immunoperoxidase staining of frozen sections and cytochemistry - thus ensuring the accurate diagnosis of this uncommon tumour.

The Histiocytoses of Childhood - Two Case Reports

James Hector - Taylor. Paediatric Department, Christchurch Hospital

Histiocytic disorders are characterised by tissue infiltration with cells of monocytic/macrophage lineage. Abnormal immune responses mediated by cytokines have been proposed as mechanisms for Langerhans cell histiocytosis (LCH) and haemophagocytic lymphohistiocytosis (HLH). It remains unclear whether the histiocytes themselves or other immune cells are the defective population.

The aetiology of LCH is unknown and affects 4 per million children each year with a peak incidence between 1 and 3 years of age. It is considered a reactive disorder resulting from immune activation.

HLH occurs in primary and secondary forms. The primary form is a genetic disorder with autosomal recessive inheritance, but the nature of the gene defect is unknown. The incidence is 1 - 2 cases per million children each year.

The diagnosis, treatment and management of two cases of LCH and HLH will be described.

Haematological Dyscrasis in the Elderly; how far do we go?

Jackie Wypych, Haematology Department, Medlab Hawkes Bay, Hastings

In every Lab we see a population of elderly patients presenting with features of dysplasia or proliferation. How often do we actively investigate and perform a plethora of laboratory tests including invasive bone marrow aspirates to achieve a diagnosis?

This presentation involves a selection of cases from a small community laboratory, where full investigations were performed on elderly subjects. Each yielded an interesting diagnosis made possible by the accessibility of cell markers, cytogenetics and cytochemistry at reference laboratories. At the end of the day what is gained? Certainly our own body of knowledge, but also we hope an effective outcome for the patients concerned.

CD56 (Neural Cell Adhesion Molecule) - The Killer Antigen

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CD56 is the 140 kDa isoform of the neural cell adhesion molecule and it is expressed on some cells of neural origin. It is also expressed by natural killer cells (NK cells) and subsets of T lymphocytes which are involved in mediating non-MHC restricted cytotoxicity.

CD56 is also known to be expressed on haemopoietic malignancies including acute myeloid leukaemia, natural killer leukaemia, non-Hodgkin's lymphoma and myeloma, and is thought to influence homing mechanisms and the pattern of malignant cell dissemination.

Case histories of 4 haemopoietic malignancies illustrating the involvement of CD56 will be presented with literature reviews examining the clinical implication of this antigen on malignant cells. The rationale for including CD56 in phenotyping all haemopoietic malignancies will be provided.

Evaluation of the Sysmex SF-3000 Automated 23-Parameter Haematology Analyser

Paul Lloyd, Haematology Laboratory, Hutt Hospital

An evaluation of the Sysmex SF3000 Haematology Analyser was performed at the Hutt Hospital Laboratory in Lower Hutt. Investigation included appraisals of precision and carryover. The correlation study was performed against the Technicon H2. The results obtained showed excellent performance for the SF3000 on all CBC parameters tested.

The SF3000 is the first Sysmex Haematology Analyser to be placed in New Zealand, and it is ideal for small to medium sized laboratories and is suited to a Core Laboratory setting.

Erythrocyte Sedimentation - a New Solution to an Old Problem

John Robert, Hitech Pathology, Melbourne, Australia

Measurement of erythrocyte sedimentation was first described 80 years ago and despite many attempts has yet to be either controlled or eliminated. There have been recent attempts to automate using either standard Westergren tubes or non-standard tubes with various algorithms to allow conversion to Westergren ESR.

A new method for deriving the ESR is described using a revolutionary device from ALIFAX S.p.A. from Italy distributed by Dade Diagnostics. This method allows a result to be obtained via closed primary tube sampling in approximately 40 seconds.

Correlations with current alternative automated methods are reviewed as well as the advantages and disadvantages of the new instrument with discussion of possible future developments.

A Mass of Masses - an Interpretation of Red Cell Mass data to determine recommendations for future testing

Terry Taylor, Healthlab Otago, DUNEDIN

Interpretation of red cell mass and plasma volume data is described. Results were collected from 57 patients (31 males and 26 females) over the past 5 years at Dunedin Public Hospital. These patients were generally having red cell mass (RCM) studies to confirm absolute erythrocytosis after initial investigations pointed toward a diagnosis of Polycythaemia Rubra Vera (PRV). There were 5 patients referred for RCM studies that were being investigated for other conditions. These patients were included in this study. Absolute erythrocytosis was confirmed in 60% (n = 34) of all cases. Equivocal results were found in 16% (n = 9) of patients, and normal to low RCM results in 24% (n = 14) of cases. None of the patients sampled showed a relative (or spurious) erythrocytosis as defined by a normal red cell mass and decreased plasma volume.

RCM was calculated from 99Tecnetium labelled blood and plasma volume (PV) from 125Iodine labelled plasma injections. Residual radioactivity in blood samples taken at regular time intervals was determined. From these readings radioactivity at time = 0 could be estimated. Final RCM and PV values were calculated using a combination of formulas from recommended sources.

The use of percentage above mean normal predicted values showed that absolute erythrocytosis was present in 38% (n = 13) of patients that had a normal measured RCM in ml/kg. These patients with a normal RCM in ml/kg had an increased mean body mass index (BMI) compared to patients who had an increased RCM in ml/kg.

Positive increases in RCM were seen over a wide range of presenting haemoglobin (Hb) and haematocrit (HCT). In males, the range for Hb was 162g/l 201g/l with a mean of 179.5 and median of 177. For females the Hb range was 147g/l 198g/l with a mean of 169.5 and median value of 170. HCT values for males were between 0.48 - 0.60 with a mean result of 0.53 and median of 0.52. Females had a presenting HCT range of 0.43 - 0.60 with a mean value of 0.51 and median of 0.50. There were no patients with a normal to low RCM presenting with a Hb or HCT value above normal reference values.

There were significant correlations between Hb and HCT values and percentage difference from predicted RCM in both male and female patients.

Regression analysis showed it was possible to extrapolate an estimate of percentage difference from predicted RCM from the Hb level in patients presenting with a Hb above reference limits. The differences between extrapolated and measured values were shown not to be significant.

Advances in Conventional and Molecular Cytogenetics: A Brighter Future for Diagnosis and Monitoring of Haematological Disease

Christine Morris, Cytogenetics & Molecular Oncology Unit, Department of Pathology, Christchurch School of Medicine

Leukaemia, lymphoma and many related haematological conditions are genetic disorders. A genetic mutation occurs in a myeloid or lymphoid progenitor cell, alters its proliferative properties, and gives rise to a clone of cells characterised by the same abnormal genetic marker. Some of these mutations give rise to microscopically visible chromosome abnormalities such as the Philadelphia (Ph) chromosome. Following the discovery of the Ph in 1960, and with the introduction of chromosome banding techniques in the early 1970s, abnormalities in the number, size or shape of chromosomes have been described in the affected cells of literally thousands of patients with haematological malignancies. These studies collectively show that different recurring chromosomal abnormalities correlate with specific clinical and biological characteristics of these diseases. Non-randomly occurring cytogenetic rearrangements are associated with distinct morphologic subtypes, and with improvement of treatment options it is evident that some of these chromosomal changes have a major prognostic value. For example, hyperdiploidy and t(12;21) are associated with very long disease free survival in acute lymphoblastic leukaemia (ALL), whereas t(9;22) and 11q23 rearrangements correlate with a poor outcome. Because of this prognostic impact, detection of these and other specific abnormalities in haematological malignancies has to be accurate and rapid at diagnosis.

Further recent developments in computer-based technologies, in combination with working reagents provided by the human genome project, have bought new and phenomenal changes to the way that cytogenetics laboratories function. Labour-intensive darkroom photography followed by manual 'cut-and-paste' preparation of karyotypes has been replaced by image-capture stations and semi-automated karyotyping software. Fluorescent in situ hybridization (FISH) has also colourised a typically black-and-white discipline, and become an essential 'molecular cytogenetic' assay for analysis of the number, size and location of specific DNA sequences in individual cells. Many past difficulties in cytogenetic interpretations are now resolvable using chromosome 'paints' or 'probes', and chromosome aberrations can be detected not only in dividing cells but also in interphase nuclei, an approach referred to as interphase cytogenetics. The prevalence of specific cytogenetic aberrations in many haematological conditions is currently being reassessed by metaphase and interphase FISH, and also by related FISH-based procedures such as comparative genomic hybridisation (CGH) and multi-colour FISH (M-FISH). New and more sensitive reagents for diagnosis and subsequent monitoring of minimal residual disease, relapse and treatment responses are inevitable outcomes of this research.

Diagnosis of Acute Lymphoblastic Leukaemia, the Importance of the Complete Picture, Presentation of a Case Study

Lynette Savage, Jill Cochrane, Rob Corbett, Ruth Spearing, Joy Monteath, Sarah Rigby, Christine Morris
Cytogenetic and Molecular Oncology Unit, Canterbury Health Laboratories, Christchurch

A case study of a 9 year old boy presenting to Christchurch Hospital with clinical features suggestive of Acute Leukaemia. Initial haematological investigation showed an increase in peripheral blood lymphocyte population with the presence of atypical lymphoblasts. Surface Marker analysis indicated the leukaemic cells expressed a preB cell (transitional) phenotype.

Subsequent Cytogenetic and Molecular analysis classified this patient's leukaemia as a Burkitt (FAB L3) Acute Lymphoblastic Leukaemia.

Monitoring of Human Blood Dendritic Cell Numbers

D Fearnley, L Whyte, S Carnoutsos, A Cook and D Hart
Presented by J McKenzie
Haematology Research Group and Department of Haematology Christchurch Hospital

Dendritic cells (DC) originate from a bone marrow (BM) precursor and circulate via the blood to most body tissues where they fulfil a role in antigen surveillance. These peripheral DC acquire antigens, and migrate via the lymph to regional lymph nodes where they initiate T-lymphocyte responses. Little is known about DC numbers in disease, although the reported increase in tissue DC turnover due to inflammatory stimuli suggests that blood DC numbers may be altered in some clinical situations. The lack of a defined method for counting DC has limited patient studies.

We therefore developed a method suitable for the routine monitoring of blood DC numbers, using the CMRF 44 monoclonal antibody and flow cytometry to identify DC. A normal range was determined from samples drawn from 103 healthy adults. The mean percentage of DC present in blood mononuclear cells (MNC) was 0.42%, and the mean absolute DC count was 10 x 106 DC/L blood. The normal ranges for DC were 0.15 to 0.70% MNC or 3 to 17 x 106 DC/L blood. This method has applications for monitoring attempts to mobilise DC into the blood to facilitate their collection for immunotherapeutic purposes and for counting blood DC in other patients. In preliminary studies, we have found a statistically significant decrease in blood DC counts in individuals at the time of blood stem cell harvest and in patients with acute illnesses, including allogeneic bone marrow transplant recipients with acute graft- versus-host disease. This preliminary data supports the hypothesis that blood DC numbers may be altered in certain disease states.

The Epidemiology of Haemochromatosis

Dr Mike Burt, Gastroenterologist, Christchurch Hospital

Haemochromatosis is a common autosomal reessive disorder of iron metabolism occuring with a prevalence of 0.2 to 0.5% in Caucasian populations. It is characterised by the excessive absorption of dietary iron and a progressive rise in body iron stores which may result in cirrhosis, diabetes, cardiac failure and arrhythmias, hypogonadism, arthritis, hepato-cellular carcinoma, and reduced life expectancy. The morbidity and mortality of haemochromatosis can be reduced by venesection to remove the iron burden, and early diagnosis is important, as the life expectancy of treated non-cirrhotic patients is normal.

The diagnosis of haemochromatosis has traditionally relied on the demonstration of increased iron stores and the exclusion of secondary iron overload. Diagnosis is therefore dependent on phenotupic expression but this is related to age and can be modified by non-genetic factors such as dietary composition, blood donation, menstruation, pregnancy and pathological blood loss. The recent identification of the haemochromatosis gene has important implications for diagnosis and screening. Originally termed HLA-H, now designated HFE, the gene encodes a 343 amino acid protein with homology to major histocompatibility class I molecules. Two missense mutations have been identified in this gene. The first clauses a substitution of cysteine with a tyrosine residue at position 282 (C282Y) and homozygosity for this mutation has been identified in 64-100% of reported patients.

Gur 1998;43:830-836

The Prevalence and Significance of Haemochromatosis Gene Mutations in the Population

Dr Ian Morison, PhD, Clinical Pathologist
Southern Community Laboratories, Dunedin

Mutations of the gene HFE are associated with increased iron absorption in the gut and are known to cause hereditary haemochromatosis. In New Zealand, the mutation which changes a cysteine to a tyrosine amino acid at position 282 (Cys282Tyr, C282Y) probably accounts for about 99% of cases of haemochromatosis, whereas in other population groups it is relatively less common in haemochromatosis patients. The high prevalence in New Zealand and Australia is attributable to emigration from the northwest European countries, especially Ireland. A Dublin study has reported that 28% of the population carried the C282Y mutation, whereas in New Zealand 14% of the population are heterozygotes. One in 200 New Zealanders are homozygous for C282Yand thus at risk of iron overload. The conservation of DNA sequences around the C282Y mutation indicates that the mutation occurred once in history, and thus it must be associated with a survival advantage.

A small proportion of individuals with haemochromatosis is not homozygous for the C282Y mutation. Other mutations/polymorphisms that might contribute to the iron overload include His63Asp (H63D) and Ser65Cys (S65C). H63D is present in about 25% of New Zealanders and S65D was detected in 4% of French patients. Both of these polymorphisms are over-represented in haemochromatosis patients who are not homozygous for C282Y, suggesting that they might also play a role in iron overload.

The Hepatic Iron Index

Trevor Walmsley, Canterbury Health Laboratories, Christchurch

The hepatic iron index is used in the diagnosis of haemochromatosis.

The liver biopsy sample is dried at 80oC overnight and 1 mg of the biopsy is digested in 500 µL of concentrated nitric acid and diluted to 5 mL. The iron concentration in the digestate is measured using flame atomic absorption spectroscopy and the iron content of the liver biopsy is calculated in µg of iron per g of dry tissue. Normal levels are 350 — 1250 µg/g dry tissue. The iron content of the liver is also expressed as an hepatic iron index, which is calculated as µmol iron/g dry weight/year of age. An index of greater than 2.0 is essentially diagnostic of haemochromatosis.

Moving molecular diagnostics into the routine laboratory

Dr Peter M. George, Canterbury Health Laboratories, Christchurch

It is now over a decade since the PCR technique was developed. Although this method greatly simplifies methods for the analysis of DNA and RNA, progress has been slow in implementing 'routine' PCR analysis. In part, this is limited by pre- and post-analytical difficulties but there are other limitations inherent in the PCR. Newer technologies are overcoming these technical limitations and it is now possible to consider point-of-care molecular diagnostics. These are likely to find early application in medical, environmental and food microbiology. However, pre- and post-analytical considerations are likely to limit the movement of 'genetic' analysis into 'routine' laboratories.

LightCycler™: real-time fluorescent PCR detection of point mutation disease – in under 30 minutes

John F. Mackay, Molecular Systems, Roche Diagnostics NZ

The LightCycler instrument combines rapid temperature ramp rates with toughened glass capillaries as PCR vessels, in order to complete amplification reactions in as little as 15 minutes. Using a fluorescent probe that spans the desired mutation, a melting curve can be performed: the amplified reaction is slowly heated and the temperatures at which the probe dissociates from the amplicon is monitored. A higher dissociation temperature indicates stable binding of the probe sequence and a lower temperature indicates a mismatch to the sequence. Multiplex applications for C282Y, H63D and S65C for haemochromatosis have been designed. Other applications such as Factor V Leiden and prothrombin will be discussed.

Proliferative Markers in Histopathology

Brett Delahunt, Department of Pathology and Molecular Medicine
Wellington School of Medicine, University of Otago

The role of the pathologist is principally one of diagnosis, however the advent of molecular biology, which permits examination of tissue at a sub-cellular level, has provided pathologists with the opportunity of deriving information regarding disease outcome and progression. The most significant advances in this area have occurred in the development of techniques that allow the quantitation of cell proliferation in fixed tissue.

Some idea as to the proliferative activity of tumours may be obtained from assessment of tumour grade and mitotic rate, although these techniques are crude, objective and subject to artefactual variation. The identification of nucleolar proteins and various antigens associated with specific components of the cell cycle has facilitated investigations into tumour proliferation as a prognostic marker.

The proliferative rate of tumours is a function of two variables; cell cycle duration and the proportion of cells within a given tumour population that are undergoing cell cycle activity.

The duration of the cell cycle within a specific tumour may be measured by staining nucleolar organiser regions using a silver-colloid technique with gold toning. There are a variety of immunohistochemical stains that label cells within the proliferative component of a tumour cell population and, of this latter group of immunohistochemical stains, Ki-67 is the most specific cell cycle label.

Quantitation of nucleolar organiser regions or Ki-67 labelling will not in isolation provide an accurate assessment of cell cycle activity, as the parameters that these markers measure are independent of each other. Cellular proliferation studies therefore must include assessment of both nucleolar organiser region score and Ki-67 index, with the proliferative activity index of a tissue being the mathematical product of these two markers.

Skin Pathology of the Elderly

Jacqui Gardner M.D., F.R.C.P.A.
Consultant Pathologist, Canterbury Health Laboratories

In this talk I will be presenting examples of some of the more common skin disorders that I see in older patients in both the hospital and the community. I will show both clinical photographs and histology slides to illustrate these cases.

Non-Formalin Fixation Methods Suited to Histology

Nina Fotinatos, Royal Melbourne Institute of Technology (RMIT) and IDEXX, Central Veterinary Diagnostic Laboratory, Victoria, Australia

Introduction:

Tissue fixation depends on a variety of factors, especially the constituents of the fixative. Formaldehyde has been the most widely used fixative for many years in histological laboratories around the world. Unfortunately formaldehyde is known to be a serious sensitisation agent which causes irritation to mucosal membranes of the eye, nose, throat and respiratory epithelium. In an effort to find a suitable substitute, alcohol-based fixatives have been hypothesised to adequately fix tissue and furthermore, not interfere with routine procedures subsequently performed on tissue.

Methods:

Formalin fixed tissue was compared to existing and newly developed alcohol-based fixatives. Existing alcohol-based fixatives are Kryofix and Spuitfix. Newly developed fixatives included Melbourne Fix 1, 2, 3 and 4. These slides were stained (H&E method) to appear similar in staining intensity and were scored by assessors. Assessors consisted of acting histologists and pathologists.

The criteria used for assessment included the following:

(1) Tissue architecture
(2) Cell borders
(3) Cytoplasm
(4) Chromatin textures
(5) Nuclear contours
(6) Uniformity of staining

Scores for assessment ranged from 1 (poor) to 4 (excellent).

Results:

Statistical analysis of all fixatives showed that comparisons between Melbourne Fix 2 and formalin consistently exhibited no significant difference. This was noted in all criteria. Melbourne Fix 2 also scored higher in Criterion 3 (Cytoplasm) and Criterion 5 (Nuclear Contours) compared to formalin.

Conclusion:

Non-formalin fixatives are safer to the handler, which should be a high priority in histological laboratories. The performance of such fixatives may be the new solution to the ever-growing problem of formalin fumes. However, it should still fix tissue adequately and most importantly appear similar in morphology. The results of this study demonstrate how Melbourne Fix 2 could be used as a substitute to formalin in histological laboratories.

Marrow Biopsies in Lymphomas and Related Disorders

R. Brunning, Department of Laboratory Medicine and Pathology, Division of Haematopathology, University of Minnesota, USA

Hodgkin's and Non-Hodgkin's B and T cell lymphomas frequently involve the bone marrow.The pattern of involvement often reflects the nature of the different types of lyphoma and these patterns will be described in some detail.

Benign lymphocyte aggregates are also a relatively common finding in bone marrow trephine biopsies. The distinction between benign lymphocytic aggregates and malignant lymphoma of small type in adult marrows may be very difficult. General guidelines for making this distinction will be proposed.

The RCPA Quality Assurance Program in Gynaecological Cytopathology — A review of six years of external QA testing

PW Shield and J Finnimore
RCPA QAP, Royal Brisbane Hospital, Herston, Brisbane

The RCPA have operated an external quality assurance program in cytopathology for Australian and New Zealand laboratories for over ten years. Since 1993 the program has been operated out of Brisbane. The content of the program has been enhanced over this period of time but has followed the same basic format of remote testing with a strong emphasis on the educational aspects of QA.

Participants complete four surveys per year for a total of 20 cases. About 200 laboratories have participated each year. A fax option allows laboratories to submit reports on the cases and obtain a rapid summary of the expected response while the slides are still in the laboratory. This feature provides an important educational aspect to the surveys.

Analysis of results obtained in the gynaecological slide surveys over the six years 1993 to 1998 reveal a general improvement in group performance, with increasing target response rates and falling major error rates for most diagnoses. In particular, cases with a target response of technically unsatisfactory and those with a target of squamous cell carcinoma have been increasingly well handled. The most common causes of difficulties over this time period have been cases with target responses of reactive/reparative changes, endometrial carcinoma and negative smears with evidence of lower uterine segment sampling. Overcalling of endocervical abnormalities in negative smears has been another common source of error.

A number of new developments are currently underway to enhance the program and make it more comprehensive. In Australia, Performance Standards for Laboratories Reporting Cervical Cytology have been under development for a number of years and provide a useful tool for statistical monitoring and feedback for both laboratories and the Screening Program as a whole. Slide surveys using fluid-based collection and preparation methods for cervical samples are also being planned. It is intended that these developments will broaden the scope of the QAP and maintain its relevance for diagnostic laboratories.

The Diff-Quik Stain in Cytology - 'Out with the old and in with the new'

Di Taylor, Cytology Department, Canterbury Health Laboratories

The MayGrunwald Giemsa stain has been used routinely in our laboratory for air dried smears. With the introduction of the Diff-Quik stain for urgent or preliminary diagnosis, the validity of the use of two separate stains was questioned.

A study was designed to compare and evaluate the Diff-Quik stain using 12 pleural fluids. The results of this study will be discussed.

Review of 100 Bronchoscopy Cases

Chris Bowden, Cytology Department, Canterbury Health Laboratories

100 patients who had bronchoscopy specimens taken at Christchurch Hospital were reviewed. By correlating the cytological diagnosis with concurrent and subsequent histological diagnoses, the accuracy of the cytological diagnosis is assessed and will be discussed.

In situ hybridisation: overcoming the lack of probes

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Centre for Advanced Biomedical Studies, School of Pharmacy and Medical Sciences
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In situ hybridisation (ISH) is a powerful and sensitive procedure, which allows the localisation of target nucleic acid sequences within chromosomes, cells or tissue sections. The technique has a molecular basis and relies on complementary binding between a single-stranded, labelled probe and a target sequence. With the development of non-radioactive probes, ISH is now being more widely applied and in particular to localise mRNA in cases where protein products cannot be detected by other means. Nevertheless a major obstacle to more widespread use of ISH is the limited number of probes available commercially — thus laboratories wishing to use the procedure need to become familiar with probe production techniques.

The preparation of probes involves several key steps:

- Designing the primers to generate the probe.
  Primers of 18-25 base pairs should be selected by extracting known primers from the literature or by 'tailor-designing' them. Probe cDNA is then made using RT-PCR which amplifies the selected region of DNA.
- Cloning (to generate sufficient probe).
  The PCR product is cloned by ligation into a vector (such as pGEM-T) and subsequently inserted into bacteria. Probe DNA is then extracted from bacteria after culture and purified.
- Sequence identification and probe labelling
  Sequencing confirms the specificity of the cloned DNA. The probe is then labelled with a reporter molecule, which can be visualised, allowing for localisation of the target sequence.

In situ hybridisation: merging molecular biology with histology

TM Nilsson and AE Woods
Centre for Advanced Biomedical Studies, School of Pharmacy and Medical Sciences University of South Australia, Adelaide, South Australia, Australia

In situ hybridisation (ISH) is a sensitive, specific and reproducible technique, which exploits the property of complementary nucleotide binding to detect a specific target sequence within a cell. The method when applied to tissue sections comprises four steps: prehybridisation, hybridisation, post-hybridisation and immunological detection. During the prehybridisation process cells are permeabilised to enhance probe entry — this is followed by hybridisation of the probe (labelled with digoxigenin, biotin or fluorescein) with the target sequence. Post-hybridisation includes stringent washes to remove non-specifically bound probe and immunological detection uses routine histochemical visualisation of the target based on hapten/antibody binding and chromogenic visualisation.

The major histological use of ISH is for the demonstration of mRNA especially in cases where:

(i) the levels of gene expression are too low for product detection by immunohistochemistry;
(ii) protein is not produced at all; or
(iii) appropriate immunological reagents are not available.

Using this technique, and probes generated and labelled in our laboratory, we have examined the expression of receptors for tumour necrosis factor (TNF)-a□ (TNFR1, TNFR2), cellular adhesion molecules (E-selectin, ICAM-1) and endothelin (ET-1, ETB) on normal and tumour-associated vascular endothelium. Thus far we have been able to localise specific mRNA to distinct vessel types within the tumour and construct a profile of receptor gene expression on tumour-associated endothelium. Our experience shows that once the probes have been made, ISH can be readily incorporated into the histology laboratory with minimal establishment needs.

New Compounds from Old Species

Clyde Riley, Women's and Children's Healthcare Network, Melbourne, Australia

A Method for Demonstrating Lectin-Like Activity In Seed Extracts From Australian Native Plants.

Lectins are proteins or glycoproteins, which have the capacity to bind sugars specifically. The majority of available lectins are derived from plants and to date there does not appear to be any published examples of lectins from Australian species.

The purpose of this exercise was to develop a means of screening seed extracts for lectinlike activity.

The use of the small vitamin biotin and its high affinity for the glycoproteins avidin or streptavidin have enabled a wide range of biologically active molecules, including lectins, to be manipulated and visualised.

Biotinyl-hydroxysuccinimide ester is relatively easy to attach to biologically active proteins and these biotinylated compounds may be visualised using variously conjugated avidin or streptavidin systems. The method can also be used to study unrefined protein mixtures.

Crude seed extracts from a number of Australian native plants were prepared biotinylated and applied to a range of paraffin-processed tissues using streptavidin HRP and diaminobenzidine as a substrate. Extracts from the species Acacia aneura bound to a number of normal epithelia and the specificity of binding was demonstrated by hapten sugar inhibition.

The method offers a simple screening process for the possible isolation of new and potentially useful lectinlike compounds.

The neuropathology of alcohol related brain damage

J.J. Kril, A.J. Harding and G.M. Halliday**
*Centre for Education and Research on Ageing, The University of Sydney, Sydney and *Prince of Wales Medical Research Institute, Randwick, Australia*

Alcohol abuse results in significant neurological impairment and neuropathological abnormalities have been identified in a number of brain regions including the cerebral cortex, diencephalon and cerebellum. However the mechanism underlying this brain damage is unclear. A number of factors including alcohol per se, nutritional deficiencies and liver disease have been hypothesised as contributing to the pathogenesis of alcohol related brain damage. Most notably, many alcoholics have a deficiency of vitamin B1 (thiamin) which results in Wernicke's Encephalopathy (WE). Furthermore, a proportion of alcoholics with WE develop an amnestic disorder, Korsakoff Psychosis (KP). We have compared the pattern of neuropathology in alcoholics with and without WE and with and without KP.

Using routine histological and immunohistochemical stains in spaced series of sections and unbiased quantitative techniques, we determined the number of neurones in the frontal association and primary motor cortices, each of the subdivisions of the hippocampal for-

mation, the anterior principal and mediodorsal nuclei of the thalamus and the medial mamillary nucleus of the hypothalamus. All alcoholics show a loss of neurons from the frontal association cortex, but no loss from hippocampal formation. Alcoholics with WE have substantial pathology in the diencephalon, but only those with KP have neuronal loss from both thalamic nuclei. These findings indicate that thiamin deficiency is important in the aetiology of brain damage in the diencephalon, while alcohol *per se* is responsible for neuronal loss from the frontal cortex.

Detection of Autoantibodies to Extractable Nuclear Antigens (Ena): Traditional versus Molecular Approaches

T.P. Gordon
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Autoantibodies to extractable nuclear antigens (ENA) are a clinically important subset of anti-nuclear antibodies, which were originally defined by immunodiffusion. Most ENA consist of complexes of a nucleic acid (DNA or RNA) associated with one or more antigenic proteins. Although the autoimmune response is generally directed against the native protein, the autoantibodies occasionally bind denatured protein. These observations are relevant to ELISA and immunoblotting where the form of the antigen may be critical for detection of the ENA. Prokaryotic expression systems in Escherichia coli are useful for producing large amounts of recombinant antigen for ELISA; these proteins may be denatured with consequent loss of the B cell epitopes. Eukaryotic expression systems including the baculovirus system produce post-translationally modified protein in native form but are expensive and too complicated for in-house production. The advantages and disadvantages of immunodiffusion, immunoblotting, immunoprecipitation and ELISA for detection of ENA are discussed.

At present, there is no ideal method, which can simply and accurately detect all of these autoantibodies, and it is often necessary for laboratories to confirm an ENA with a second or third test. A newer approach is to stably transfect mammalian cells with a CDNA encoding the antigenic protein. Cells hyperexpressing the protein can be used to increase the sensitivity of detection of antibodies to ENA of low abundance such as Ro(SSA).

Are Tissue Slides of Any Use in Detection and Confirmation of Autoantibodies?

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The HEp-2 cell, and more recently the transfected HEp-2000 cell, has largely replaced tissue slides for the detection of antinuclear antibodies. However, tissue sections are still useful for the confirmation of certain autoantibodies, and are necessary for the detection of many types of autoantibody. This presentation will discuss the use of various tissues in the detection and identification of autoantibodies associated with a variety of autoimmune diseases.

IgG Subclasses in Clinical Immunology. Are we any wiser?

Prof Antonio Ferrante, Department of Immunopathology, Women's and Children's Hospital, and the Department of Paediatrics, University of Adelaide, South Australia

Subclasses of human IgG were first mentioned by S. Dray (Science 1960; 132:313-4) almost 40 years ago. Since then there have been many efforts to understand the clinical relevance of IgG subclass measurements. While new and significant developments occurred during each of the last four decades, there has been continuing controversy with respect to assay methods, standardisation of the technology, definition of an IgG subclass deficiency and the clinical significance of a subclass deficiency.

Human IgG consists of IgG1, IgG2, IgG3 and IgG4, which have relative serum concentrations of 70%, 20%, 6% and 4% respectively. These vary greatly in biological properties. IgG1 and IgG3 in contrast to IgG2 and IgG4 are strongly opsonic and complement fixing. It is important to measure IgG subclasses even when the total IgG is normal since a decreased level of one subclass can be compensated by an increase in another. The type of antigen, eg. protein, or carbohydrate, usually dictates the type of IgG subclass antibody response to the antigen, and failure to produce normal levels of antibodies to certain antigens may indicate a deficiency of a specific subclass. Deficiencies in all subclasses, either as isolated or combined deficiencies, have been reported. While deficiencies in isolated IgG subclasses have less dramatic consequences than total IgG deficiency, serious and life threatening infections can be experienced by patients with these deficiencies. Measuring of IgG subclasses should be carried out where there is a history of recurrent, prolonged or severe infections. The type and site of infection may be an indicator of a specific type of IgG subclass deficiency. For example in isolated IgG2 deficiency the individual fails to make antibodies to encapsulated bacteria and may experience recurrent respiratory tract infections with pneumococci or *Haemophilus influenzae* Type B. The most rewarding aspect of screening for IgG subclass deficiencies is the ability to treat the condition with replacement immunoglobulin.

The measurement of IgG subclasses has helped to define primary and secondary immunodeficiency syndromes better and has added to our understanding of antibodies which protect against IgE-mediated allergy. It has now also been realised that IgG subclasses to autoantigens could be used as indicators of risk of damage in autoimmunity as was recently shown for diabetes in relation to the autoantigen, glutamic acid decarboxylase. Since IgG subclass patterns are influenced by cytokine patterns associated with T helper 1 and T helper 2 lymphocytes, IgG subclass measurements may be useful markers of the activity of T cell subpopulations.

In the last five years the major progress in the IgG subclass field has been in the standardisation of technology for quantitating IgG subclasses eg. automated equipment and the development of age-related normal ranges. Consequently we are now in a position to re-evaluate and extend our understanding of the clinical significance of measuring IgG subclasses. The treatment of IgG subclass deficiency is best instituted in early childhood when normal concentrations are low hence automated technology together with concerns about the definition and clinical significance of IgG subclass deficiency, continues to provide a challenge to the immunologist.

Internal Quality Control of Indirect Immunofluorescence Assays

W. K. Pollock, Gribbles Pathology, Immunology Department, Melbourne, Australia

The RCPA QAP Immunology Working Party recently undertook a sur-

vey of all laboratories participating in the Immunology programme regarding quality control (QC) of indirect immunofluorescence (IIF) assays.

The aim was to examine ways in which the current QC guidelines required for accreditation purposes by the National Australia Testing Authority (NATA) could be improved upon. As many of these NATA guidelines were written some time ago, they do not necessarily reflect the technological changes that have recently taken place.

Results of the sixty laboratories that responded were summarised and presented for discussion at the Immunology QC Workshops at Bond University in May this year.

Proposals for internal QC in IIF have been drafted as minimal and optimal recommendations. This is to take into account the difference in expertise, number of samples, patient populations and the use of in-house assays. It is expected that all laboratories perform the minimum recommendations but be encouraged to perform the optimal ones.

These draft proposals have been circulated to all participating RCPA QAP Immunology laboratories and will be presented for discussions at the meeting along with the results of survey.

It is anticipated that these proposals will be finalised and presented to NATA for consideration by the end of the year.

Advances In Fighting Influenza

Dr Lance Jennings, Virology Section, Canterbury Health Laboratories

Influenza remains a threat to public health worldwide. Its success as a pathogen is largely because of its ability to undergo antigenic variation, resulting in annual epidemics and from time to time, pandemics of disease associated with high morbidity and mortality. The control of influenza is at present based on the use of vaccines aimed at preventing influenza caused by the influenza A and B viruses most likely to circulate in the following influenza season. Annual influenza vaccination is effective in protecting individuals at high risk of severe influenza and its complications. In the elderly, all respiratory illness is reduced by 56%, pneumonia by 53%, hospitalisation by 50% and mortality by 68%

The cost benefits of immunisation extend to low-risk individuals, including health-care workers and other healthy adults. Efficacy is greater after repeated annual vaccination, and side effects are minimal. International influenza surveillance is an integral part of this strategy for maintaining antigenically current vaccines and detecting new potential pandemic viruses. As the natural reservoir for influenza A viruses are aquatic birds, surveillance for avian and other animal viruses has gained in priority. With the new generation of antivirals, the neuraminidase inhibitors zanamivir and oseltamavir, the therapeutic potential in combating both influenza A and B virus infections now exists.

House Dust Mite and Cat Allergens in the Antarctic

R Siebers, P Weinstein, P Fitzharris, J Crane*
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Allergens of the house dust mite (HDM) and domestic cats provoke asthma and rhinitis in sensitised individuals. Areas not conducive to HDM proliferation and free of cats should be allergen free. To test whether HDM and cat allergens can be passively transferred from clothing into allergen-free areas we have measured these allergens at Scott Base, New Zealand's permanent Antarctic Base on Ross Island.

We obtained vacuumed dust samples from three living-room areas and twelve mattresses at Scott Base. Additionally, dust samples were obtained from the jerseys of eleven recently arrived Scott Base personnel, seven of who kept cats in their homes in New Zealand. The dust samples were analysed for the major group one HDM and cat allergens, Der p 1 and Fel d 1 respectively, by double-monoclonal antibody ELISA techniques and expressed as µg of allergen per gram of dust.

Der p 1 was undetectable in the three living-room areas and only detectable in one mattress (0.61 µg/g). Six jerseys had low Der p 1 levels ranging from 0.28 to 2.18 µg/g. Fel d 1 was detectable in one living-room area (0.44 µg/g) and in seven mattresses (range: 0.43 to 1.49µg/g). Ten jerseys had Fel d 1 levels ranging from 0.45 to 4.18 µg/g.

This study confirms passive transfer of cat allergen into a remote and cat-free environment. The levels of Fel d 1 found could result in unexpected symptoms in cat-allergic individuals due to the aerodynamic properties of cat allergen.

Age related infectious disease management

Dr Larry Reimer, Prof. Pathology & Medicine Head, Section of Clinical Microbiology
Director, Clinical Microbiology Laboratories and Infectious Diseases, Utah, USA

Presentations of common skin, respiratory tract, and genitourinary tract infections in the elderly will be presented. In a format similar to the congress workshop on cost effective microbiology, laboratory protocols with the most benefit in diagnosis of these common conditions will be discussed with time for the audience to interact about their own experiences and approaches to appropriate laboratory utilisation.

Application of *M.tuberculosis* DNA fingerprinting in New Zealand

R.H. Vaughan, A.A. Vaughan, A.J. Morris, M.C. Croxson
LabPlus, Auckland Healthcare, Auckland

The aim of this study is to describe molecular typing of *M.tuberculosis* (M.tb), introduced as a collaborative study between the New Zealand Tb Reference Laboratory, Green Lane Hospital, and the Virology/Immunology DNA Department, Auckland Hospital. Molecular typing has been developed to assist in outbreak recognition, to identify laboratory cross-contamination, and to assist in the differentiation of reactivation versus re-infection with M.tb.

Our method uses the repetitive and random insertion of IS6110 as a basis for DNA fingerprinting. This is in accordance with the standard reference method used worldwide.

We report the use of this technology to resolve possible laboratory cross- contamination, the analysis of three putative outbreaks of M.tb, and the contribution it has made to epidemiology. Immediate Public Health benefits resulted, including the unexpected identification of geographically widespread contact cases.

The results indicate that this is a reliable, reproducible method for typing M.tb isolates. We conclude that significant advances in the control of M.tb may be anticipated by collating the typing patterns of all M.tb isolates in New Zealand.

Pigs, pole-toilets, and pek-pek - a parasitologist in paradise

Wayne Melrose, School of Public Health and Tropical Medicine
James Cook University, Townsville, Australia

A light-heartened look at medical science in the land of the unexpected - Papua New Guinea, where being chased by pigs, falling into toilets, and taking blood samples from ex-head hunters in the dead of night is all part of the fun. The paper will also outline of the health challenges faced by this developing nation and the part that Australian and New Zealand Medical Scientists are playing in meeting them.

Studies Of *Campylobacter* Isolates in New Zealand, 1995 - 1997

C M Nicol and J Wright, Institute of Environmental Science & Research Ltd, Porirua, New Zealand

Campylobacteriosis is the most common cause of gastroenteritis in New Zealand (228.5 notified cases/100 000 1996). From 1995-1997, several typing studies were performed using human and non-human isolates. The aim was to determine the usefulness of various typing methods and the prevalence and distribution of types in New Zealand.

Methods evaluated were: serotyping (Penner), biotyping (Preston and MAST-ID), mRFLP (macro-restriction fragment length polymorphism using pulsed field gel electrophoresis and *Sma*I). All isolates were serotyped and typed by at least one other method.

No one method was ideal: 17% of isolates did not serotype; Preston biotyping was labour intensive and difficult to standardise; MAST-ID was easier to set up but difficult to read; mRFLP was erratic in the quality of results, 11% were untypable. Of five hundred human isolates typed, the following serotypes were the most common: 2 (24%); 4 (11%); 1 (6%); 11 (6%); and 8 (4%). Campylobacter indistinguishable by two typing methods can be found in humans, poultry, cows, sheep and untreated water. Background data from the studies were used in subsequent outbreaks to identify potential outbreak strains. The variety of methods and their inadequacies make implementation of a regular typing programme difficult. International standardisation of methods would be useful.

The Role of Alloiococcus otitidis in Otitis Media: Results of a Pilot Study

John Aitken, Mr Mark Ward, Dr Peter Thornley*
**Southern Community Laboratories, The Princes Margaret Hospital, Christchurch*

There is a high incidence of otitis media in New Zealand under the age of 6. For the last two years, this laboratory has carried out studies on antibiotic susceptibility patterns of respiratory pathogens isolated from the nasal secretions of children presenting to General Practitioners. These studies have highlighted significant carriage rates of beta lacramase positive respiratory pathogens in local children.

In order to investigate the likelihood of an association between organisms isolated from nasal secretions, and those concurrently present in middle ear effusions, we carried out extensive bacteriological investigations on routine samples taken from 10 patients presenting for grommet insertion.

Of the 10 samples, 3 grew bacteria. Four of the samples grew only a single isolate, while the other four grew a mixture of organisms. The most common isolate was Alloiococcus otitidis (6 patients). Alloiococcus otitidis has been isolated from the middle ear in several overseas studies and is gaining respect as a probable agent of infection in chronic otitidis media.

otitidis has unusual cultural requirements sometimes associated with growth in biofilms. Successful isolation is moderately difficult, and may explain why A. otitidis has not been isolated in New Zealand.

Lymphatic filariasis: new strategies for diagnosis and control

Wayne Melrose, School of Public Health and Tropical Medicine James Cook University, Townsville, Australia

The mosquito-borne parasitic disease lymphatic filariasis infects around 120 million people and is classified by the WHO as the world's second most common cause of long term disability. The parasite causes a wide spectrum of disease. Many cases are asymptomatic but it has recently been shown that even in these cases lymphatic and renal damage is occurring. Other patients may suffer recurrent episodes of fever (often mistaken for malaria) which may be accompanied by acute lymphangitis, and in males, inflamation of the scrotal contents. Repeated attacks can lean to chronic lymphodema and the gross limb and scrotal deformities known as elephantiasis. Traditionally diagnosis was by the demonstration of microfilariae in night blood. This method is inconvenient to both patient and investigator and because not all cases are microfilaraemic, up to 30% of cases may be missed. The use of filarial antigen tests has revolutionised the diagnostic process. Blood can be taken at any time and both microfilaraemic and amicrofilaraemic cases will be detected. Effective, low cost treatment regimes consisting of an annual dose of Diethylcarbamazine (DEC), Ivermectin, Albendazole, or a combination of these drugs have brought the eradication of filariasis within reach as can be demonstrated by the success achieved in parts of Papua New Guinea.

Alternative to the thick blood film for the Detection of low-density malarial parasitaemia

Wayne Melrose, Sarah Paget, Pauline Dixon, Richard Speare
School of Public Health and Tropical Medicine
James Cook University, Townsville, Australia

The Becton Dickinson QBC, ICT rapid card test, Cell Labs antigen ELISA and DNA probes were compared to the traditional thick film for the detection of asymptomatic low-density parasitaemia in 268 Papua New Guinean secondary school students. The QBC test is more sensitive than the thick film but costly and species identification is a problem. The ICT test was found to be rapid, specific and sensitive and suitable for large batches or single samples. There were no false negatives but there is a problem with persisting antigen after treatment has cleared the parasitaemia. The Cell Labs ELISA gave comparable results to the ICT test but is more complicated. DNA probes are useful for species confirmation but are usually only available in reference laboratories. The performance of the thick film was surprisingly good and is perfectly adequate in situations where the high cost of the other tests is a consideration.

Leishmaniasis

Anna Ruddenklau, Canterbury Health Laboratories

In 1997 a Dutch tourist presented to a Christchurch GP with two large ulcers on the hand and elbow. Following consideration of recent travels in South America he was referred to the Infectious Diseases Service at Christchurch Hospital. The differential diagnosis of Leishmaniasis was favoured and histological and microbiological investigations of biopsies were performed. Confirmation of this diagnosis was achieved using PCR analysis in Columbia, South America.

This presentation follows the development, diagnosis and

treatment and resolution of this parasitic disease as experienced by the tourist. The three main forms of Leishmaniasis, mechanisms of establishing infection, their geographical distribution and clinical features are discussed, as is the difficulty associated with diagnosis.

Recent Advances in Urine Culture

Dr Galiano, Alifax, Florence, Italy and Dr Enzo Breda, Alifax, Florence, Italy

Dr Breda is the inventor of the instrument URO-QUICK. In his curriculum, several patents appear in the field of automation in microbiology and coagulation.

He has conceived and carried out the kinetic systems for the instrument URO-QUICK, patent US N5.356.815 of 18/10/94, the quick system for the RAA (Residual Antimicrobial Activity) and is developing further devices to optimise the instrument. His latest inventions are TEST1 and MicroTest1, revolutionary analysers for the determination of the ESR, automated and manual methods respectively.

His researches have been utilised by private companies as well as hospitals. Just the closest to us are quoted here.

He was born in Udine, north of Italy, in 1944 and took a degree in Nuclear Chemistry in 1968 at the University of Padova. He took a diploma in Computer Science at IRCA in Milan in 1971, and is taking a degree in Medicine and surgery at the University in Udine.

He was a researcher at ENI (SNAM ORIGETTIS) 1669
Director of the Research Laboratory LEPETITI Pharmaceuticals (Milan) 1971-1972

Director of the Clinical Chemical Analyses Laboratory SALUS in Udine 1973 1990.

Crossmatching the Blood Donor Database: The Utilisation of ABS Census Data for Donor Recruitment in Victoria

Mark Meade and Patrick Coghlan, Australian Red Cross Blood Service Victoria, Melbourne

As we strive to become more strategically focused in our approach to donor marketing the need to use hard data and statistics becomes more apparent. The Australian Bureau of Statistics provides census data for the 1996 census, and this is accessible using three commercial software products, which provide quite sophisticated analyses for application to donor marketing.

ARCBS-Vic utilise the software package 'Map Info' to 'crossmatch' the current Melbourne metropolitan donor database of 120,000 donors with the 1996 census data. The software allows donors to be grouped according to their socio-economic and demographic profiles and a map of where donors live relative to the collection centre where they donate can be produced. This allows qualitative deductions to be made about the dominant profiles of donors attending particular mobile venues, as well as from where they have been recruited.

The statistical content behind this map provides a very useful tool for donor recruitment. From the 'crossmatch' of the donor database with the ABS census data we have been able to measure the propensity of donors to donate blood according to the socio-economic and demographic profile to which they belong. Eligible donor populations can be estimated for any particular area thus allowing for more informed planning of donor drives and ultimately more effective donor recruitment.

Applying AC Nielsen data, which captures local community profiles, we can further refine this approach to developing effective donor recruitment strategies for each locale. This presentation demonstrates the utilisation and effectiveness of 'crossmatching' the blood donor database with census data.

Intravenous Immunoglobulin Use in Southland

Sue Baird, Southland Hospital, Invercargill

Intravenous immunoglobulin (IVIG) use in Southland is reasonably high given our population. We purchase approximately 2.5 times the amount of IVIG we are allotted under the Plasma Entitlement System (PES).

To determine the disorders being treated with IVIG a retrospective search of diagnosis codes was made on patients for 1 year (1998). This data will be shown.

Many conditions have a well-documented positive response to treatment with IVIG, these include Idiopathic Thrombocytopaenia Purpura (ITP), Kawasaki's Disease, Guillian Barre' and Myaesthesia gravis. A brief description of these will be given.

Very little information on the mode of action of IVIG is readily available, however, some mechanisms are known:

(a) inhibition of complement binding and prevention of MAC formation;
(b) neutralisation of some pathogenic cytokine;
(c) downregulation of antibody production; and
(d) modulation of Fcreceptor mediated phagocytosis.

Curiourser and curiouser

Sheryl Khull, MidCentral Blood Service, Palmerston North

Initially this appeared to be an ordinary antibody identification panel containing anti-D. However, the patient's history brought the origin and identity of the antibody under question.

The patient is a 74 year old man with a long list of medical conditions, including bladder cancer and hypertension, and now a positive direct antiglobulin test. He had recently been transfused with three units of Rh(D) Negative blood, all from well known Rh (D) Negative blood donors, and had not had any platelets or plasma products.

The possibility that this was an autoantibody was considered. Elution and autoabsorption techniques yielded no useful information. Samples were submitted to the Red Cell Serology Laboratory at New Zealand Blood Service's Northern Centre and the International Blood Group Reference Laboratory in Bristol, England. They confirmed the presence of anti-D in the serum and the patient's red cells to be of normal D Negative, rr phenotype.

Consultation via the American Association of Blood Banks bulletin board yielded suggestions that were followed up. These investigations revealed that one of the 'Rh Negative' donors had a weak D antigen. Molecular characterisation of the donor's cells classified her as a Weak D Type 2. This is the first published example of de novo stimulation of anti-D by a weak D antigen. It has implications for defining requirements for efficacy of antisera used for D typing blood samples, especially those of blood donors.

91/2 Weeks Gestation — Titre 1024

Amanda Hayward (Waikato Blood Transfusion Services, Hamilton)
Jim Faed(Dunedin)

Problem:
The K antigen, K 1, is a strong immunogen; its antibodies can cause

severe haemolytic transfusion reactions and HDNB. An antenatal patient presented as a referral from the private laboratory: 'First antenatal screen, atypical antibody detected for identification.' Mrs P was only 91/2 weeks pregnant.

Methods:
Antibody screens and panels were performed by enzyme and IAT methods. Titres performed by saline IAT each time, titring the previous specimen in duplicate to allow for technical variation. Red cell antigen typing performed to antisera manufacturer's specifications.

A sample of amniotic fluid was sent to IBGRL, Bristol, England for PCR amplification, to assess Baby P's Kell genotype. IBGRL use sequence specific primers to amplify exon 6 of the Kell gene which is where the point mutation occurs that gives rise to the Kelleher/Cellano polymorphism.

Results:

91/2 weeks	Anti-K detected	titre 1024
12 weeks	titre. 2048	Mr P's genotype Kk
13 weeks	amniotic fluid sample sent to IBGPL. Baby P's genotype kk	
24 weeks	titre 1024	
28 weeks	titre 1024	
32 weeks	titre 1024	
40 weeks	edd 8699	

Conclusion:
This was an interesting case in that international technology was used in the investigation of a possible severe case of HDNB. IBGRL has an accuracy rate of greater than 99% using this technology. Baby P has not been delivered yet so the 'proof of the pudding' will come when the 'chicken has hatched'. The technology is now being developed in Auckland to identify Rhesus genotypes.

Post Script:
By August, Baby P will have been born so a final conclusion to this case will be presented.

Computer Cross Matching (C-XM)

Erolia Eteuatis, Waikato Hospital, Hamilton

Background:
A Computer cross-match (C-XM) is an electronic verification of ABO and RhD compatibility between donors and patients who do not have clinically significant antibodies. The Waikato Hospital Blood Bank (BB) decided to investigate C-XM because of the number of journal articles advocating its benefits.

Study Design and Method:
In February 1998, our Blood Management System was upgraded and it had the facility to do C-XM. Prior to this, there were no adequate warning systems in place. For example, one could quite easily issue Group B blood to a Group A patient without any warnings being given. There were several criteria that had to be met before the implementation of C-XM. These included supplier documentation, software validation, group confirmation of donor units, two blood group results on the patient, a negative antibody screen result, and sample results that were current.

Results:
The software validation process confirmed that C-XM could be done safely. The other key requirement was to familiarise staff with its use. Training was organised, and once competence records were signed off, staff were allowed to C-XM.

Conclusion:
With the introduction of C-XM, the Waikato Hospital BB workflow has improved significantly, particularly in the busy periods and when staff are working on their own. It is a process we would recommend to any Blood Banking service.

When an 'A' is not an 'A'

Lucinda Masson, NZ Blood Service, Christchurch

An 'A' is not an 'A' when it is a *cis* AB. My talk outlines how we encountered this rare blood group and the changes it forced us to make in our donor blood grouping procedure. I will also briefly cover the genetics behind the blood group.

Conditioned Cryoprecipitate

Suzanne Williams, NZ Blood Service, Christchurch

Conditioned cryoprecipitate is used for the correction of a low plasma fibrinogen. A standard dose was 6 units, after the conditioning process the yield of fibrinogen from a single donation has increased to allow a standard dose to be lowered to 4 units. Thus lowering the number of units to which the recipient is exposed.

The conditioning process does not require any special equipment, just a freezer controlled to maintain temperature at -5C.

Parachute Packing and Life Raft Inflation

John Aitken, SCL, Christchurch
Ben Harris, SCL, Christchurch
Mike Southern, Biolab Scientific, Christchurch
Jan Parker, Health Funding Authority, Dunedin

Four viewpoints presented by Medical Laboratory Scientists who elected to be refugees from the public health laboratory system.

Each speaker will briefly outline their personal employment journey (before and after working in the hospital environment). And provide some insight into employment prospects in the wide world. The speakers have been asked to share some insights and provide some direction for other medical laboratory scientists contemplating a similar journey.

Medical Laboratory Accreditation Standards; Past, Present and Future

Graham Walker, International Accreditation New Zealand

Since July 1994, International Accreditation New Zealand has required accredited medical testing laboratories in New Zealand to be fully compliant with ISO Guide 25 - General Requirements for the Competence of Calibration and Testing Laboratories. Accordingly, annual internal audits, formal management reviews, active investigation of complaints and the implementation of effective corrective action etc. have become well established quality improvement tools in all accredited medical testing laboratories.

ISO Guide 25 has itself been the subject of recent review and major revision, and the new version, to be issued as a full international standard entitled ISO 17025, is expected to be published late in 1999.

Both ISO Guide 25 and ISO 17025 however, have primarily defined the requirements for calibration laboratories, and both represent a 'poor fit' for medical testing laboratories. This 'poor fit' has in part been responsible for reluctance among medical testing laborato-

ries internationally, to implement the requirements of either standard.

In recognition of the special needs of medical testing laboratories, ISO contracted NCCLS to develop on its behalf, a quality management and technical performance standard specifically for medical testing laboratories. Numerous meetings at various locations throughout the world, each with significant and detailed input from the pathology industry, have resulted in the development of a new international standard entitled ISO 15189 Quality Management in the Medical Laboratory.

ISO 15189 incorporates all those elements of pre-existing international standards including ISO 9002 and ISO 17025, relevant to medical testing. In addition, it adds a unique medical interpretation for some of the irrelevant, ambiguous or difficult to interpret clauses of pre-existing standards. ISO 15189 is a standard developed by the pathology industry, for the pathology industry.

The implementation by International Accreditation New Zealand of ISO 15189 as the accreditation standard for medical testing laboratories in New Zealand is expected to follow the publication of the standard early in 2000. The ramifications of this change in the accreditation criteria document are explored.

New vs Old — A comparison of the Training of Medical Laboratory Technologists

SE Cooper, NZ Blood Service, Northern Region, Auckland

The modern day Medical Laboratory Science qualification has undergone great changes since its conception years ago. A comparison of life as a Medical Laboratory Student in the 90s, compared with that of technologists trained 15 — 20 years ago, reveals some interesting findings. In this presentation we will take a look at qualifications, technology and study — how much has changed?

Also, a comparison will be made of the BMLS degrees currently being offered in NZ by three tertiary institutions. A look at how well they are preparing students for the reality of the working laboratory, and how they can be improved. This paper discusses these issues and raises important questions as to which is the better training method for this degree — the old way or the new?

"Are you ready to Master Applied Science? Experience of a current student"

H.Perry, Auckland Institute of Technology, Auckland, NZ

In 1999, I entered into the new Masters of Applied Science programme at Auckland Institute of Technology.

The first year of the programme consists of a series of modules, which can be combined to allow for individual interests. An appropriate theoretical basis for research is developed and compulsory modules develop competence in managing the research process and writing grant applications. Specialist modules address aspects of cell biology, chemistry and environmental science.

The skills developed in the first year of study equip the second year student to undertake an in-depth investigation in a specialist area of research. In this second year the student will be assigned to a postgraduate academic supervisor who guides them in the development of research skills and thesis preparation. Established active researchers supervise student research.

The objectives of the programme are to develop not only an excellent research graduate but also an individual who can integrate easily into the work environment and whom industry will actively seek.

Bachelor of Medical Laboratory Science (BMLS) provides an entry path to this degree.

For more information, please e-mail kiwi@ait.ac.nz

Keeping Cardiac Patients Out of Hospital

M. Gary Nicholls, Physician, Department of Medicine
Christchurch Hospital, Christchurch

The costs of managing heart failure in most Western countries including New Zealand accounts for more than 1% of total health care budget. Less than 20% of this cost is accounted for by pharmaceuticals and over 70% comes from hospitalisation. Beyond budgetary concerns, the outlook for patients with established heart failure is bleak, and quality of life is poor. Both the incidence and prevalence of heart failure in New Zealand is increasing.

Prevention of heart failure is dependent on first, early diagnosis and treatment of essential hypertension, second, prevention of coronary artery disease, and third, efficient institution of measures to limit myocardial infarct size and prevent left ventricular remodelling after acute infarction.

Cardiac natriuretic peptides, particularly BNP and NTproBNP, are proving excellent prospects as a biochemical guide to diagnosis of heart failure, as a guide to prognosis in patients with established heart failure, and a guide to pharmacotherapy. BNP levels rise in the early phases of heart failure, and can be used to differentiate heart failure from other causes of shortness of breath, particularly airways disease. Cardiac natriuretic peptides have also been shown to give a prognosis in frail elderly subjects, and in patients after acute myocardial infarction. Local studies in progress suggest that plasma BNP might be used as a guide to intensity of antiheart failure therapy.

Optimal management of heart failure requires a team effort involving GP, cardiologist, biochemistry laboratory, nurse, dietitian, radiology and nuclear medicine. Hospitalisation rates can be reduced substantially by a planned coordinated approach. In that the problem of heart failure is an increasing one and in view of costs involved, a team approach is logical. This should also have a research and development component to take advantage of local research expertise. Linkage with pharmaceutical companies for early access to new therapeutic agents is logical.

Integrated care: the laboratory perspective.

Dr Peter M. George, Canterbury Health Laboratories, Christchurch

Integrated care requires communication, and the sharing of information, between all of the parties involved in the care of a patient. This should be a two-way process, with laboratories receiving information from clinicians and others involved in the care. This is likely to require an information technology approach, with integration of clinical, pharmacy, radiology, laboratory and financial systems from the private and public sectors.

Laboratories also have a responsibility to provide appropriate turnaround times, to support point-of-care testing and to help develop clinical pathways. This will include appropriate laboratory initiated testing. It is also important that laboratories and clinicians work together to demonstrate outcomes based on testing. As new tests are introduced, the traditional evaluations should be extended to include an assessment of their effect on clinical practice. Although this will require a partnership approach, it will probably need to be led from the laboratory.

Examples from cardiovascular medicine will be used to illustrate the process.

Primary Calibration in Clinical Chemistry — A Personal Perspective

John Kitto, Southern Community Laboratories

The presenter draws on 40 years experience in Clinical Chemistry to review the issue of Primary Calibration from a Quality Management perspective and takes a refreshingly unconventional personalised approach to the subject.

The paper raises the conventional questions on the subject of Primary Calibration such as source and frequency but proceeds to cite reference texts and personal models of Quality standards, which are often overlooked addressing this subject.

The author draws widely on his personal experience, putting the present pre-millennial scene in a historical perspective before challenging contemporary scientists to examine their own approach to the future.

The emphasis is on the holistic rather than the analytical approach to the subject, the paper citing attitudinal, communication and teamwork as equally significant factors as matrix, source and traceability in the overall context of Gold standard Calibration.

Personal experience in managing this process is presented along with a summary of practical tips for those seeking to gain experience in this area of clinical chemistry.

The paper concludes with a challenge to all those in charge of sections or departments within the wider laboratory scene and summarises this approach as

Leadership
Goals
Priorities
Material

This paper should have wide appeal not only to the dedicated Scientist in Clinical Chemistry as it applies equally to all disciplines. It fits neatly into the context of the theme for the Congress - "The ageing process" being the reflections of an active but senior scientist summarising his conclusions.

Pseudohypertriglyceridaemia

Trevor Walmsley, Canterbury Health Laboratories, Christchurch

A patient was referred to our lipid clinic for investigation; analysis of their plasma showed a low cholesterol level with a raised triglyceride. However, the high triglyceride level was not consistent with the clear plasma sample (pseudohypertriglyceridaemia).

Lipoprotein electrophoresis showed a normal lipid pattern and again was not consistent with the high triglyceride measured in this sample. At this stage we examined the chemistry of our triglyceride method and determined that the method measured both free glycerol and glycerol from triglyceride after hydrolysis with lipase. An alternative method was used that measured free glycerol without interference from triglyceride. The free glycerol level was measured on this sample and was found to be raised. Correction of the triglyceride level in our patient for free glycerol showed that the triglyceride level in this patient is normal and consistent with the plasma appearance and electrophoresis results.

Markedly low levels of glycerol kinase were found in the patient's leucocytes. The pseudohypertriglyceridaemia is due to a high level of glycerol as a result of glycerol kinase deficiency. These results explain why this patient's triglyceride levels have been resistant to treatment. Since our findings, the patient has been taken off all lipid-lowering drugs,

The measurement of urine glycerol, as a diagnostic aid in identifying these patients will be discussed.

Serum Methylmalonic Acid (MMA) by Isotope Dilution; a Marker for Vitamin B12 Deficiency

Chris Leaver, Specialist Biochemistry, Canterbury Health Laboratories

The usual laboratory tests for determining whether a patient is deficient in vitamin B12 (or Cobalamin) is to measure total serum B12 levels, blood haemoglobin and to assess red blood cell morphology. However total serum cobalamin does not always reflect the concentration in tissues and anaemia is only seen in approximately 60% of patients with cobalamin deficiency1. There are reports of patients with low serum cobalamin without cobalamin deficiency and reports on cobalamin deficient patients with serum cobalamin within the health related reference range.

L-methylmalonyl CoA is an intermediate in the biochemical pathway from propionyl CoA to succinyl CoA. Precursors of propionyl CoA include isoleucine, valine, threonine, methionine, cholesterol and odd chain fatty acids. 5'deoxyadenosylcobalamin is an essential cofactor in the enzymatic conversion of L-methylmalonyl CoA to succinyl CoA by methylmalonyl CoA mutase. Since there are no alternative pathways, the methylmalonyl CoA that accumulates in cobalamin deficiency is hydrolysed, and methylmalonate accumulates in the blood.

Serum MMA has been found to be a useful diagnostic test for functional cobalamin deficiency in the tissues. It has been used overseas for more than 10 years, but measurement in New Zealand has not been available due to the requirement for a gas chromatograph mass spectrometer (GCMS). A brief overview of the stable isotope dilution method currently being set up in Christchurch will be given along with some preliminary results.

Reference:

1. How to diagnose cobalamin deficiency. Ebba Nexo, Mads Hansen, Karsten Rasmussen, Anders Lindgren and Ralph Grasbeck. Scand J Clin Lab Invest1994;54 (suppl 219) 61.

Vitamin B12 and Folate Assays on Elecsys 2010

John Sheard, Biochemistry, Coast Health Care, Greymouth

Vitamin B12 and Folate were the largest volume sendaway tests from our laboratory and we had been interested in performing them in-house for sometime, but did not have suitable instrumentation available. Reagents for Vitamin B12 and Folate for the Roche Diagnostics Elecsys 2010 were released in late 1998. This paper describes our evaluation of the performance of these reagents with emphasis on:
a comparison with results from our sendaway provider quality control results compared to Roche Diagnostics claims reference ranges

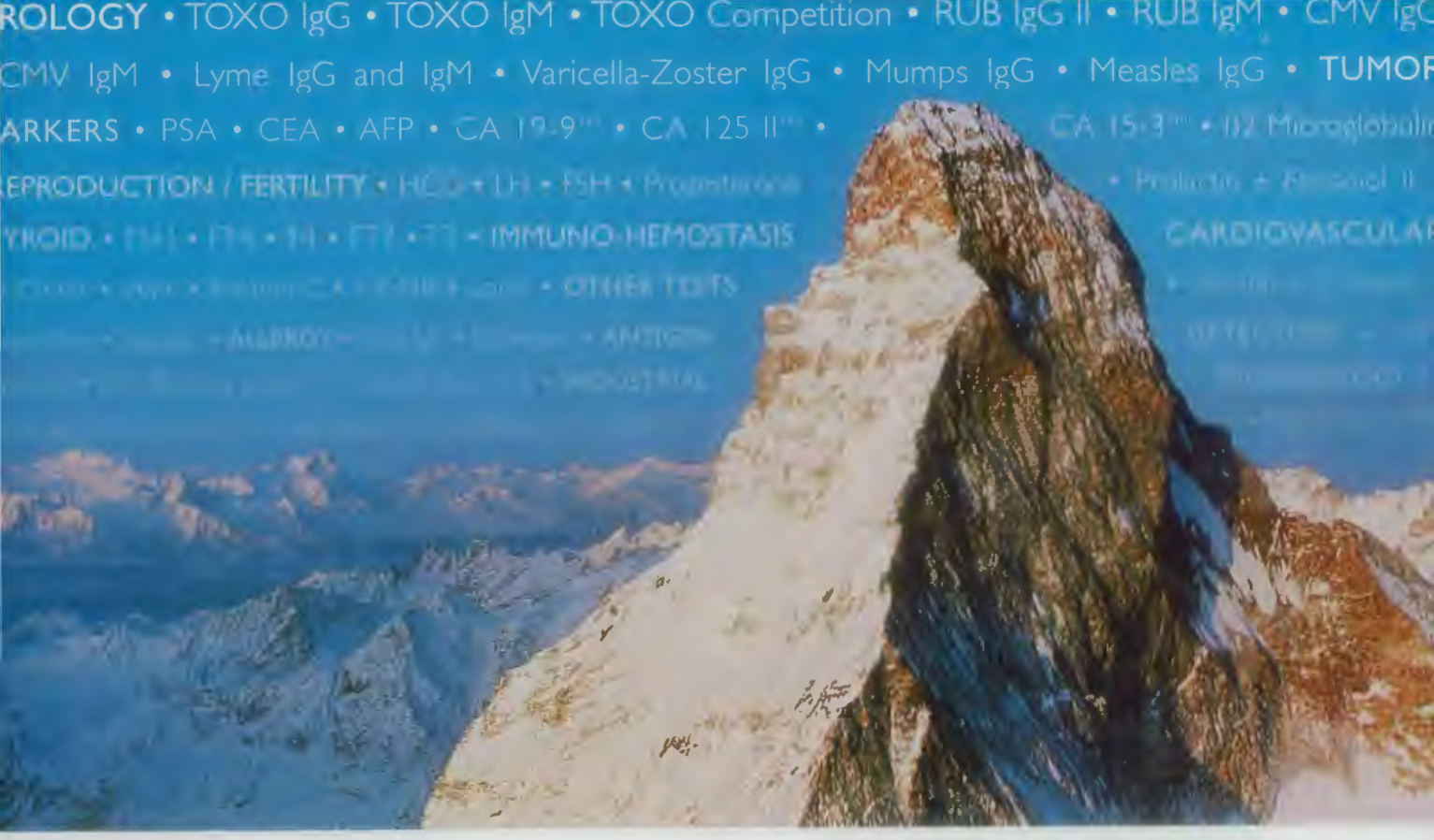
There is considerable variation in published reference ranges for Folate and information supporting our choice will be presented.

The evaluation showed that the assays performed satisfactorily and they have been in routine use in our laboratory since April 1999.

Core Laboratories; the Royal Hobart Hospital Experience

N. Byron, R. David, Royal Hobart Hospital, Hobart, Australia

Core laboratories of differing models have been put forward as one possible method to increase efficiency of pathology laboratories, thereby providing a mechanism to deal with the increasing demand and contracting budgets facing the pathology industry.



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The pathology departments of the Royal Hobart Hospital (RHH) have changed from separate discipline based departments headed by a medical director and a scientist in charge, to one department of pathology, headed by a director and a scientist in charge. The department has been divided into functional units headed by a scientist deputy manager. The Core laboratory is one such functional unit.

The Core laboratory combines routine biochemistry, haematology, coagulation, transfusion medicine, and near patient testing. This change has been accompanied by a move in premises to a new purpose designed laboratory, new automation and has involved multiskilling and regular rotation of level 1 scientific technical staff. This has allowed consolidation and uniformity in work flow and work practices. The core laboratory was preceded by other functional changes such as a single pathology request, combined specimen reception, and IT.

The change to a core laboratory service at the RHH although not without problems has been successful. However not all laboratories are suitable candidates for this process and the RHH experience is offered here only as an example of the processes involved.

Possible future directions include workstation consolidation to incorporate other automated areas such as serology and immunodiagnostics. Twenty-four hour operation is planned, however staff resources need to be reallocated as staff vacancies occur to progress this. The core laboratory concept should also allow for future flexibility with staff roles and staffing mixes.

Molecular Genetics in the Haematology Laboratory

Dr Ian Morison, Southern Community Laboratories, Dunedin

Molecular genetics currently plays a significant role in the diagnosis and management of haematological malignancies. The current use of DNA-based studies in the leukaemias and lymphomas will be reviewed. In particular molecular techniques are allowing subclassification of the leukaemias into clinically significant risk categories. The emergence of new powerful techniques for the detection of molecular changes will have profound effects on the laboratory of the future. For the haematopoietic malignancies, techniques including automated PCR and RT-PCR, assay-based comparative genomic hybridisation, and spectral karyotyping will revolutionise our approach to diagnosis and management.

Current developments suggest that molecular techniques will also have a great impact in the coagulation laboratory. An increasing number of gene polymorphisms are known to influence an individual's risk of venous thrombosis. Similarly, polymorphisms affecting platelet function can predict an increased risk of early thrombotic arterial disease. I predict that automated PCR-based genetic profiling, perhaps based on DNA-chip technology, will lead to population screening and prophylactic therapeutic interventions in those at high risk of premature thrombotic disease.

Current and Future Directions of Bone Marrow Transplant Laboratories

Annette Trickett, BMT Laboratory, Haematology, St George Hospital Gray St, Kogarah, NSW 2217, Australia

In the early days of bone marrow transplantation (BMT) there was little need for a dedicated BMT laboratory. By the 1980s, the advent of autologous BMT as a viable treatment option for patients lacking a suitable donor created the need for laboratories with specific expertise in cryopreservation and cell culture. The role of the BMT laboratory has since expanded to accommodate the changes in clinical transplantation. BMT has become a recognised treatment option for patients with haematological malignancies, aplastic anaemia, haemoglobinopathies, metabolic disorders, and immunodeficiencies, and is being evaluated for patients with solid tumours and autoimmune diseases. Haematopoietic stem cells are now obtained not only from bone marrow, but also from peripheral blood and cord blood. Each stem cell source presents further technical challenges: timely harvest of peripheral blood stem cells requires accurate methods of monitoring of blood stem cell counts, and cord blood cells require more stringent cryopreservation protocols. Many laboratories are also required to deplete T cells from allogeneic transplants in an effort to reduce the incidence and severity of graft-versus-host disease, and select stem cells from autologous transplants to reduce the number of contaminating malignant cells. The transition of the BMT laboratory from experimental to routine cell processing has necessitated formulation of quality assurance guidelines and accreditation. Possible future directions of BMT laboratories include (1) ex vivo culture of haematopoietic stem cells, (2) gene therapy, and (3) immunotherapy.

Comparisons between CD 34 cell counts and the Immature Reticulocyte Fraction for Stem Cell Mobilisation

J. Cohen, D. Rosenfeld, L. Dunlop
Haematology Department, South Western Area Pathology Services, Liverpool Hospital, Liverpool, NSW, Australia

For patients who require having their peripheral blood stem cells harvested for re-infusion purposes at a later date, they must first undergo a regime where chemotherapy and G-CSF are administered. The objective is to attempt to mobilise production of CD34 positive stem cells until the levels of CD34 positive cells are high enough to be harvested. The patients have frequent pre-harvest screening tests performed to verify the absolute CD34 cell counts until the cell count reaches 15 x 106/L. These tests are an FBC, Reticulocyte count and the Immature Reticulocyte Fraction (IRF), as well as a CD34 cell count.

The Reticulocyte count is performed on the *Cell Dyn 4000* at the same time as the FBC is processed. The technologies for performing the reticulocyte count utilise both fluorescent flow cytometry along with intermediate light scatter at 7°. The IRF is determined as the fraction of reticulocytes that exceeds a predetermined level of RNA fluorescence.

Data from 35 attempts at stem cell mobilisation will be presented, and the findings show that the Immature Reticulocyte Fraction has a valuable role to play as part of the pre-harvest screening procedure. Some of the pre-harvest screening tests show a low IRF, these also had very low CD34 cell counts. It is proposed that the IRF could be used as an initial screen to determine if a CD34 cell count needs to be performed. This could lead to financial benefits to the laboratory, as fewer CD34 cell counts would be required.

Regulation of Migration of Blood Dentritic Cells

Justin Roake and Colin Hagan
Department of Surgery, Christchurch School of Medicine

Dendritic cells are crucially important in initiating immune responses. To function, they must migrate within the body via well-defined pathways. We have shown that a distinct set of molecular signals (mediated via chemokines) regulates migration of blood dendritic cells.

Clinical Aspect of Lupus Coagulant

Dr David Heaton, Haematology Consultant, Christchurch Hospital

The lupus anticoagulant is one of the antibodies found in the so-called antiphospholipid syndrome, which may present as a primary disorder or secondary to another illness or drug exposure. Despite the name, it is often not associated with systemic lupus erythematosus and is more commonly associated with thrombotic complications than with bleeding problems.

Cases from the author's clinical practice, which exemplify some of the many forms of presentation of this syndrome, will be presented and summarised. Although the adverse effects of this syndrome are due to an autoantibody, immunosuppression is not usually helpful and treatment usually involves the use of anticoagulants.

Technical Aspects of Testing for Lupus Anticoagulants

Dr Thomas Exner, Gradipore Ltd, Sydney, Australia

Recent improvements in testing for lupus anticoagulants (LA) have come about through the availability of more specialised test methods, reagents, references, instrumentation and following the analysis of several multicentre laboratory surveys.

Screening tests vary widely in LA sensitivity. Tests are also variably affected by platelets and abnormalities other than LA. Thus mixing tests and phospholipid correction procedures are recommended, but most of all, a knowledge of the limitations of each type of test method is important for achieving a correct laboratory diagnosis of LA.

Possibly the most difficult preanalytical variable is control of platelet microparticles which bypass weaker LA and shorten some clotting tests. Double centrifugation or filtration of test plasma is often useful, but may be ineffective in patients with thrombotic processes in vivo. Several test systems are relatively unaffected by therapeutic anticoagulants such as heparin and oral anticoagulants, though mixing with normal plasma can correct any deficiencies and usually provides the most reliable result. The normal plasma used in such mixing tests also needs to be platelet/phospholipid poor. Some clotting machines yield test results quite different from those achieved manually, especially with abnormal plasmas and these results may need to be carefully normalised for valid interpretation or compared with a more appropriate reference range.

The specificity of some LA test systems remains of concern, with two recent studies suggesting a high incidence of LA among haemophiliac patients. To account for differences between various LA tests some researchers have suggested that two main subtypes of LA exist and that these may require different plasma cofactors (beta 2 glycoprotein 1 and prothrombin) for their expression. Monoclonal antibodies against specific epitopes on these phospholipid-binding proteins do function similarly to LA derived from patients in clotting tests and may be useful as laboratory reference materials. However, they may not be representative of the pathologic antibodies actually responsible for the prothrombotic complications associated with LA (and antiphospholipid antibodies).

Coeliac Disease: A New Zealand Perspective

H Bramwell Cook, MB, FRACP, Gastroenterologist, Gastroenterology Department, Christchurch Hospital, Christchurch

Coeliac disease (CD) may be regarded as the great mimic of today. It has many faces with myriad presentations, and may go unrecognised throughout life. CD is defined as a permanent gluten sensitive enteropathy. There is a genetic predisposition to CD (HLA DQw2 present in nearly 100% of coeliacs), an environmental factor gluten (wheat, rye, barley and possibly oats), and probably also an as yet unidentified trigger factor or factors. The mucosal small bowel injury caused by exposure to gluten is believed to be immune mediated. Characteristic small bowel histology includes crypt hyperplastic villous atrophy with a lymphoid infiltrate in the lamina propria and a high density of intraepithelial lymphocytes. Histologic improvement is seen when gluten is withdrawn from the diet.

The IgA antiendomysial antibody (EMA) has high specificity and high sensitivity (90%) for CD. Tissue tranlutaminase (TTG) has been shown to be the autoantigen for EMA. Anti-TTG antibodies may also be recognised in CD. The toxic prolamins of the cereals are rich in glutamine and proline. TTG cross links glutamine rich prolamins and may be involved in the presentation of antigen in the immune mediated injury that occurs in CD. Antigliadin and antireticulin antibodies are also found in CD but are both less specific and less sensitive for CD than EMA.

Case acquisition studies have shown prevalence figures between 1:3700 to 1:500. Four studies in healthy blood donor populations have shown prevalence figures between 1:250 and 1:340. Two studies in children and two in adults have shown prevalence figures between 1:83 and 1:198. A study in Christchurch has shown the prevalence of CD to lie between 1:82 and 1:177. These studies show that the true prevalence of CD is considerably higher than that determined by case acquisition studies and suggest that for every coeliac that is found, there are several who go through life unrecognised. In Canterbury there has been, since the mid-1980s, a steady increase in the number of CD subjects recognised each year. Females outnumber males especially when recognised in the 2050 year age range, but not when young or old.

In children growth retardation, abdominal distension and diarrhoea are common. In adults malabsorption may cause diarrhoea, anaemia with iron/folate deficiency, malnutrition, and bone mineral loss with an increased risk of fractures. There is strong association with autoimmune related disorders such as type 1 diabetes (510%) and ulcerative colitis, and also with Down's syndrome (1015%). Whereas in early years the consequences of malabsorption were the focus of attention, much more subtle presentations are now being recognised. For some, the major symptom is lethargy and tiredness, often without any gastrointestinal symptoms.

While adherence to a strict life-long gluten free diet is not without difficulty, the improvement in well-being experienced by many makes it most worthwhile.

Data from the Christchurch Coeliac Disease Prevalence Study and illustrative cases will be presented.

Rickettsia

K Ameara, LabPlus, Auckland

The Virology and Immunology Department at Auckland hospital became involved in testing on a patient who had recently returned from Queensland, Australia and was experiencing fever with rigors, myalgia, rash, sore throat, and vomiting. Initial differential diagnosis included infection by Dengue virus, Ross River virus (RRV), or Rickettsia (query *R.australis*).

After exclusion of Dengue and RRV, the testing of acute and convalescent sera by IFA supported recent rickettsial infection, most probably due to a species in the spotted fever group. The findings

were substantiated by an external (Australian) laboratory specialising in rickettsial serology.

Both the clinical presentation and timing of antibody responses were consistent with rickettsial infection. This case demonstrates that rickettsial infection, can be acquired by individuals visiting Australia and, rickettsial serology should be considered as part of a differential diagnosis in patients (a) presenting with similar symptomology, and (b) who have recently visited an area where they have sustained multiple tick bites.

Earlier Detection of Acute Hepatitis C Virus Infection by an Automated Chemiluminescent Enzyme Immunoassay

J.A. Diment, C. Chandler, G Colebrooke, P. Pashby, M. Brown, A. Brockas, P. Niven, S.R. Lee
Ortho Clinical Diagnostics, Chalfont St. Giles, Bucks, UK and Raritan, NJ

A fully automated chemiluminescent enzyme immunoassay for the detection of antibodies to hepatitis C virus (HCV), was developed for the Vitros™ Immunodiagnostic System. The assay utilises HCV recombinant antigens c22-3, c200 and NS5 immobilised on a plastic well and a monoclonal antibody specific for human IgG conjugated to enzyme. All sample and reagent handling is automated. Results are calculated from a stored positive calibrator signal. Clinical sensitivity was assessed using serial specimens from 12 individuals undergoing seroconversion following HCV infection.

The Vitros ECi Anti-HCV assay detected anti-HCV earlier than a currently licensed EIA in 8/12 (67%) of cases. Low to medium antibody level samples gave a 1.7 to 7 fold higher sample to cut-off ratio in the ECi assay compared with the comparative assay. Specificity in blood donors was 99.76 % (n=5374). The coefficient of variation on reactive samples measured at two trial sites averaged 8.3%. All genotypes tested reacted equivalently in the assay.

Conclusion:
The Vitros ECi Anti-HCV assay combined excellent sensitivity and specificity with the advantage of a rapid, automated random access immunoassay system.

Cost Accountable-patient Outcome Oriented Clinical Microbiologic Procedures

F.P. Koontz, Professor Pathology, Director, Clinical Microbiology Laboratory University of Iowa Hospital, USA

In this era of "downsizing" or rightsizing" of hospital laboratories, many labs are starting to curtail their menu of tests offered. For the inhouse patient with underlying disease (eg leukemia, lymphoma, etc) these limitations can be organised or structured, but for the outpatient, the reverse might be true. It may well be better to increase the same menu to save further dollars down the line for the outpatient. The direct detection of Group A beta Strep by Antigen capture may cost the lab more but save dollars ultimately. For an inpatient with Strep throat, the less expensive, more sensitive culture method is more cost effective. Do we really need antibiotic susceptibility tests on isolates from a UTI? Most patients are put on empiric therapy with TrimSulfa anyway. Inhouse patients may have anything as the microbial culprit in a UTI, while out-patients have more predictable isolates with more predictable antibiotic patterns in their UTI, but do UTI isolates need susceptibility tests routinely or only in specific cases.

The concepts of specimen rejection or limited work-up must be extended to include sputum, wound and other specimen types including blood cultures. We must be aware of the problems of 'overworking' cultures mixed with non-significant flora from the skin or adjacent mucous membranes, or transitory bacteremias in blood cultures, etc. Labs must ensure that specimen tests (eg. latex antigens) can yield misinformation as well as data already known.

How do we properly work up patients for diarrhoeal disease? In-patient procedures are under strict limitation controls but not outpatients. How can the individual physician best utilise the lab with the respective patient load? What are the limits for outpatient stool cultures? How many, for how long?

The value of the direct specimen Gram Stain cannot be over emphasised. Better than half of the antibiotic therapy can be markedly focused by a good Gram Stain interpretation. Similarly as good, Gram Stain can detect a poor specimen, which shouldn't even be submitted for culture. Thus, the stain is an invaluable screening test and should be utilised as such.

An Outbreak of *Clostridium difficile*-associated diarrhoea; Microbiology helps Infection Control

Toni de Lautour, Tairawhiti Healthcare Laboratories

An outbreak of C. difficile-associated diarrhoea (CDAD) occurred over a sevenmonth period, where the majority of case patients were over the age of 60. One patient's death was attributed to complications caused by CDAD and another patient developed acute renal failure.

Because of the seriousness and highly infectious nature of the disease, control of the outbreak was obviously important. Communication between the Infection Control Coordinator, the Wards and the Microbiology Laboratory and prompt laboratory diagnosis was essential in identifying positive cases to aid in applying rational implementation of Contact Precautions.

The laboratory diagnostic test used (detection of C. difficile Toxin A by latex test) was reviewed by literature search and found to have acceptable sensitivity and specificity.

No specific source or mode of transmission of the organism was proven to be the cause of the outbreak, however there was a series of breaches in infection control precautions identified. Risk factors in the outbreak included:

Antibiotic therapy The majority of cases had received antibiotics, with many of these patients having received ceftriaxone (used widely in this institute for treatment and surgical prophylaxis).

Surgery

Patient-care equipment

Cohorting and transferring of patients

Environmental cleaning

Prevention of further cases of nosocomial CDAD in this hospital involves ongoing monitoring and prompt notification of positive tests by Microbiology, and infection control auditing. The Infection Control committee has also instigated the development of an antibiotic policy to encourage rational antibiotic prescribing.

Some Aspects of the Aetiology of Diarrhoea in Children from the Canterbury Region

Tom Henderson, Charge Technologist, Microbiology Laboratory Medlab South, Christchurch

New Zealand has acquired a reputation for leading the developed world in the incidence of bacterial and protozoal diarrhoea. If that is

truly the case then the putative capital is said to be Christchurch. Additionally it has been reported that children are the most often at risk and have the highest incidence per age group especially for Campylobacter.

At Medlab South, the faeces samples submitted from children under 5 years old were routinely tested for the presence of Salmonella, Shigella, Campylobacter, Yersinia, Aeromonas and Giardia. In response to developments occurring overseas, an acidfast stain for the detection of Cryptosporidia and a sorbitol McConkey plate for the detection of E. coli 0157 were added to our testing protocol for a period of one year (1995). A total of 1169 samples were examined during the course of twelve months in an effort to determine the isolation and occurrence of the common pathogens of diarrhoea in this age group.

Leucocyte Adhesion Deficiency — A Case Study

L Graham, LabPlus, Auckland, New Zealand
K Kelly, LabPlus, Auckland, New Zealand

Case study of a now 30 month old female who presented at birth with multiple skin lesions and a neutrophilia. Swabs of the lesions showed a heavy growth of Staph. aureus. At 4 months there was still a persistently elevated leucocyte count with a neutrophilia. Delayed separation of the umbilical cord was noted, healing and new pustular lesions were present. Further investigations included the demonstration of the complete absence of CD11b and the partial absence of CD18. A Neutrophil Chemotaxis Assay was also performed.

Neutrophil Chemotaxis Assay:
Fresh neutrophils are harvested
Chemoattractant (f-met-leu-phe) is added to wells in a chamber
Membrane layered on (pore size = 2 microns)
Neutrophils added
Incubated, filter stained
Numbers of neutrophils passing through the filter are assessed

A normal chemotaxis response against f-met-leu-phe in our laboratory is greater than 50 neutrophils per high power field (phf). In this case a result of 9 neutrophils (phf) was obtained and on repeat testing 2 months later the result was 5 neutrophils (phf).

The markedly abnormal chemotaxis assay in conjunction with the deficient CD11b/18 neutrophil markers is consistent with the diagnosis of Leucocyte Adhesion Deficiency.

Methylmalonic Acidaemia - Necessity For Continued Treatment.

C de Luen, D Webster, C Wilson, Auckland Healthcare, New Zealand

The methylmalonic acidaemias (MMA's) are a family of disorders in the metabolism of branched chain amino acids in which activity of methylmalonyl-CoA mutase is defective. These disorders can be divided into mutase apoenzyme defects, which do not respond to Vitamin B12 (B12) or defects in cofactor synthesis or cobalamin metabolism, which do respond to B12.

Previously the Health department in New Zealand had fully funded B12 therapy but it is now necessary to demonstrate the patient is B12 responsive. We tested two patients with methylmalonic aciduria for their response to B12 after intramuscular injection (IM).

KL was a 20 year old woman who presented as a neonate. B12 response had been tested in the past but was inconclusive. JT was a 13 year old girl who presented in a coma in April 1997 with a tentative diagnosis of MMA. The patients had been off B12 for at least two months. Two urines were collected on separate days then each patient was given IM B12. A further two urines were collected and all assayed for methylmalonic acid by gas chromatography. Results are below.

Methylmalonic acid mmol/mmol creatinine		
	Pre B12	Post B12
KL	0.21, 0.28	0.25, 0.26
JT	4.34, 3.06	3.85, 1.49

KL appears to be unresponsive to B12. The results for JT are inconclusive and will be repeated.

Alphafetoprotein Control Material Problems in the Changeover from Hafp Delfia Kit 1244-004 to Hafp Kit A004-201

M. Stuart[1], D. Webster[1], B. Knox[1] and T. Halonen[2],
Laboratory Services Auckland Healthcare, Auckland1, EG&G Wallac Finland[2]

Introduction
With the introduction of hAFP kit A004-201(dry kit) as a replacement for kit 1244-004 (wet kit) we had expected to changeover very smoothly but came across an unexpected problem. Our control material was not behaving in the same way with the new kit A004-201. We have been using J&J 2nd Trimester controls since we first started running our Maternal serum screening program. They have been very stable over this time. Control values fell 15% with interbatch precision showing up to 17%CV.

Results
1. We checked out and discounted the possibility of damage during shipment.
2. Patient values showed no change when wet and dry kits were compared.
3. MoM's from these assays showed no shifts.
4. We then imported the Dual hAFP/b□hCG. Our values showed a return to their previous values. Patient values did not show the same increase.
5. External QA program results were the same for all three kits.

Conclusion
Based on the data there must be something in the matrix of the controls that interferes DELFIA assay causing the difference in their behaviour compared to patient samples and the high between run variation. Why there is so much difference between wet and dry plates might have something to do with the kinetics.

A manufacturer recommended commercially available control which is checked when any changes are made to kit specifications would ensure that this problem did not re occur.

Training for the Future: The Role of the Pacific Paramedical Training Centre in Medical Laboratory Technician Training in the Pacific

M. Lynch, J. Elliot, R. Mackenzie, C. Murphy, C. Story

Pacific Paramedical Training Centre, Wellington, New Zealand

Training of Medical Laboratory Technologists in the Pacific Island nations has progressed significantly over the past fifty years, from the time when there were only a few fully trained personnel, to the present, when there are many training opportunities offered both in-country and by a variety of countries in the region.

The Pacific Paramedical Training Centre (PPTC) was established on the Wellington Hospital campus in 1981, its mission to provide teaching and development programmes which are appropriate, affordable and sustainable for the health care setting in which they will be used. The emphasis has been on short-term practical training in the various disciplines of medical laboratory science, which will ensure immediate benefit in the trainees work setting.

In 1991 the Centre became a Collaborating Centre of the World Health Organisation, a status which carries with it a responsibility for a range of medical laboratory programmes which include training and laboratory development, a regional quality assurance programme and advisory services.

This presentation defines the training philosophy of the PPTC both past and future and discusses the impact of training courses, advisory services and the quality assurance programme on the quality of health laboratory services in the Pacific Island nations.

Clinical and laboratory aspects of two recent tiger snake bites.

Morling A, Moraes C, Ruscitto M, Thom J, and Baker R
Royal Perth Hospital, Perth, Western Australia

The western tiger snake, Notechis ater (scutatus) occidentalis, has long been recognised as one of Australia's deadly snakes. Tiger snakes were responsible for 8 of the 30 snake bite deaths recorded in Australia from 1981 to 1994. We describe two cases of snakebite, which highlight clinical presentation, coagulation disturbance and the specificity of venom identification.

In common with most of Australia's dangerous snakes, there is usually a period of hours before a tiger snake victim suffers serious or irreversible deterioration normally due to progressive neurotoxin induced paralysis with or without phospholipase A2 induced rhabdomyelolysis. One of the cases we describe caused immediate collapse requiring resuscitation, adrenaline and ventilation.

Tiger snake venom has a strongly pro-coagulant action, which in vivo produces defibrination with raised D dimers but not thrombosis. However the coagulopathy is often less profound and more rapidly reversed after antivenom therapy than in taipan and brown snake bites. Factor assays performed on samples from our two patients demonstrated depletion of factor V, to levels of <5% and less extreme depletion of factors VIII, IX, X and XI. Non linearity when samples were diluted for factor IX and XI assays also indicates the presence an inhibitor, the nature of which has not been elucidated.

Snake venom detection tests on one patient post antivenom therapy gave positivity in urine, most strongly with taipan, then with tiger and weakly with brown snake. A bite site swab on the same patient reacted strongly for tiger snake with no cross reactivity.

The rapid collapse of our patient could be attributed to a high dose of venom or an anaphylactic response by this snake handler, sensitised by previous exposure. The mechanisms causing factor V depletion and non linearity in factor assays need further investigation and if due to venom components may prove of use in monitoring unneutralised venom or as a reagents for coagulation research. The reliability of a snake venom detection kit result obtained from urine needs to be established.

A Novel Test for Identifying Complement Component Deficiency

Mark van Voorthuizen, Immunology Department, Canterbury Health Laboratories

Complement deficiencies are rare, genetic disorders involving absence of one or more complement proteins. Deficiency generally predisposes an individual to serious bacterial infection or to autoimmune disease.

Haemolytic (CH50) assays are used to assess integrity of the classical complement cascade: component deficiency manifests as an inability of patient serum to lyse antibody-coated erythrocytes. Haemolytic activity serves as a screening test only, without identifying the deficient component. A full analysis necessitates quantitation of each component by RID or other method — a generally laborious and expensive task.

We have developed a simple, rapid test to identify component deficiency using a technique (CIE: counterimmunoelectrophoresis) routinely employed in Immunology laboratories. The test requires addition of complement antisera to separate wells of an agarose gel. Patient serum, added to a nearby trough, is electrophoretically driven towards the wells. Reaction of complement components with their respective antisera generates precipitin arcs, which can be visualised by staining the gel. Absence of an arc suggests absence of the corresponding component. Confirmation of deficiency requires a quantitative assay for the component in question, with no need to further tests the full range of components.

To B*lot* or not to B*lot*; that is the question?

A.R. Margaritis, C.R. Seed & T.J. Cobain
Australian Red Cross Blood Service - North West Region (ARCBS-NWR) Perth, Western Australia

Introduction:
Whilst safety for blood recipients maybe our primary responsibility we also have a 'duty of care' to our donors to responsibly counsel and follow up in the event of a positive test result. Traditionally confirmation of screening assay reactive samples for HIV and HTLV antibodies has relied primarily on Western Blot (WB) assays. These assays continue to be plagued by non-specific reactions. They also lack sensitivity when compared with the current third generation Enzyme Immunoassays (EIA) and Nucleic Acid amplification techniques. Approximately 70% of HTLV and 20% of HIV samples reactive in screening assay will yield 'Indeterminate' results on current commercial WB assays. This results in many blood donors suffering needless anxiety and being excluded as donors despite a lack of apparent risk factors or other serological evidence of disease.

Methods:
All blood donor samples are initially tested for HTLV I/II and HIV 1 & 2 on Abbott PRISM Chemiluminescent Immunoassay (Abbott Diagnostics, North Chicago). All HIV PRISM repeat reactive samples are tested using Genelavia Mixt HIV 1 & 2 EIA (Sanofi Pasteur, Marne, France). All HTLV repeat reactors are tested by both Serodia HTLV I Passive Particle Agglutination (PPA) (Fujirebio Inc, Japan) and Murex EIA (Murex, Dartford UK) for HTLV I/II. Any samples reactive on either or both of the second line supplementary assays are tested by WB to determine their final serological status.

Results:
In 1998 we screened 101 137 donations of which 26 were HTLV I/II

repeat reactive. Of these 26, 2 first time reactors were reactive on Serodia PPA, but neither on Murex EIA. Subsequent WB testing on these 2 donors resulted in indeterminate results.

For the same time period we had 68 HIV 1 & 2 repeat reactive samples of which 6 first time reactors were also Genelavia reactive. The 6 new reactors included 1 true positive, 3 WB Negatives and 2 WB Indeterminates. All of the other repeat reactors were assumed to be false positives and not counselled.

Discussion:

Our results demonstrate that by adopting a strategy based on the use of carefully selected 'second line' assays prior to blotting we can minimise the number of indeterminate results. The second line assay selection criteria focuses on comparable sensitivity and separate false positive populations. This strategy is both effective in detecting true positives and is cost efficient.

A Comparison of the 'Gradileiden V' and 'APC Resistance V' Kits for the Detection of Factor V (Leiden) defect

D. Zebeljan, M.A. Stacy, D Rosenfeld, SWAPS, Liverpool, Australia

This study presents a comparison of 2 kits used for Factor V (Leiden) (FVL) screening: 'Gradileiden V' (Gradipore), a RVVT based assay and 'APC Resistance V' (IL, Beckman-Coulter), an APTT based assay. FVL was confirmed by PCR for all patients.

The IL kit uses citrated plasma pre-diluted in factor V deficient plasma (included in the kit), while the Gradipore kit does not use a pre-dilution step. Both kits were performed on a Behring Coagulation System (Dade-Behring). The results are expressed as the ratio (APCR) of the clotting time with activated protein C (endogenous [Gradipore] or added [IL]) to the clotting time without activated protein C.

A total of 55 patient samples were tested: 25 normal, 28 heterozygous and 2 homozygous for the FVL defect; 32 were anticoagulated; 42 patients were tested for lupus anticoagulant (4 positive, 4 equivocal and 34 negative).

The APCR normal range was 1.5 – 3.9 (Gradipore) and 1.9 — 2.6 (IL) (n=25). FVL positive plasmas had an APCR range of 0.7 – 1.4 (Gradipore) and 0.7 – 1.6 (IL) (n=30).

Using a cutoff of 1.5 for Gradipore kit and 1.9 for IL kit, both kits detected all FVL samples. Both kits appeared to be insensitive to the presence of anticoagulant therapy or lupus anticoagulants.

This study showed that the Gradileiden V kit was able to detect FVL patients without having to pre-dilute samples in factor V deficient plasma.

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de Luen C, Webster D, Wilson C., Auckland Healthcare, New Zealand

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Two cases of imported Bancroftian filariasis in North Queensland

Wayne Melrose, Sean Murray-Smith, Kevin Zischke, Jean Covey
School of Public Health and Tropical Medicine
James Cook University, Townsville, Australia

Bancroftian filariasis is a mosquito-borne disease caused by the filarial worm *Wuchereria bancrofti*. The parasite causes a wide spectrum of disease. Many cases are asymptomatic but it has recently been shown that even in these cases lymphatic and renal damage is occurring. Other patients may suffer recurrent episodes of fever (often mistaken for malaria) which may be accompanied by acute lymphangitis, and in males, inflamation of the scrotal contents. Repeated attacks can lean to chronic lymphodema and the gross limb and scrotal deformities known as elephantiasis. Filariasis used to be endemic in Queensland and Northern New South Wales until the 1950s. This poster describes two cases of imported filariasis occurring in Papua New Guinean school children being educated in North Queensland. Both had microfilariae in their blood and were capable of infecting local mosquitoes. These cases illustrate the need for filariasis to be considered as a diagnosis when investigating fever in patients from tropical areas and stresses the need for vigilance lest previously controlled diseases re-emerge in Australia.

Filariasis in Travellers and Expatriates - an under diagnosed disease?

Wayne Melrose, Paul Turner
School of Public Health & Tropical Medicine, James Cook University, Townsville, Australia

Thousands of American servicemen became infected with Filariasis during the Second World War and a number of cases of filariasis among missionaries, expatriate workers and travellers have been reported in the literature. Despite this, filariasis is seldom entertained as a diagnosis when such people present with an illness. Presenting symptoms can be diverse unexplained fevers, malaise, musculoskeletal symptoms, acute lymphangitis, chronic lethargy and depression have all been reported. Diagnosis can be difficult, as most cases are not microfilaraemic. Appropriate clinical history, demonstration of high titre antifilarial antibodies and response to therapy all strongly suggest that the patient is suffering from filariasis.

Gliadin and Tissue Transglutaminase Antibodies in Patients with Increased Transaminases

Pollock W, Deam D, Eckert D, Taylor R, Gribbles Pathology, Melbourne, Australia

It is now recognised that clinically overt cases of coeliac disease represent only a small proportion of cases with this disease. There are many more patients with silent or latent forms of the disease. A paper by Volta et al showed that 9% of patients with cryptogenic hypertransaminaseaemia are affected by symptom free coeliac disease.

As the finding of isolated elevated transaminases is not uncommon in our patient population we investigated a selected group of ninety patients who had isolated transaminase increases for gliadin and tissue transglutaminase antibodies.

Methods

Ninety patients who had isolated elevated transaminases levels were selected from routine samples referred to our laboratory. Patients who had a known possible cause of their elevated transaminases (from clinical notes or previous test results) were excluded from the study.

IgG anti Gliadin antibody was measured with a QUANTA Lite Gliadin IgG Elisa method.
IgA anti Gliadin antibody was measured with a QUANTA Lite Gliadin IgA Elisa method.
IgA anti transglutaminase antibody was measured with QUANTA Lite tTG (Tissue Transglutaminase) IgA ELISA method.

Methods were performed as described by the manufacturers.

Results

Of the 90 patients, 17 (18.9%) were positive for IgG Gliadin Antibodies, 10 (11.1%) were positive for IgA Gliadin Antibodies and 4 were positive for IgA tissueTransglutaminase Antibodies.

Of the positive results for IgG Gliadin Antibodies, 5 were also positive for at least one other antibody; for IgA Gliadin Antibodies positive results, 4 were also positive for at least one other antibody; and for IgA tissueTransglutaminase Antibodies positive results, 3 were also positive for at least one other antibody.

Two patients were positive for all 3 antibodies.

The incidence of positive values was statistically higher than that for the general population.

Conclusion

The finding of positive values was statistically higher in the elevated transaminase patients than amongst the general population.

Coeliac disease should be considered in the differential diagnosis of isolated hyertransaminasaemia.

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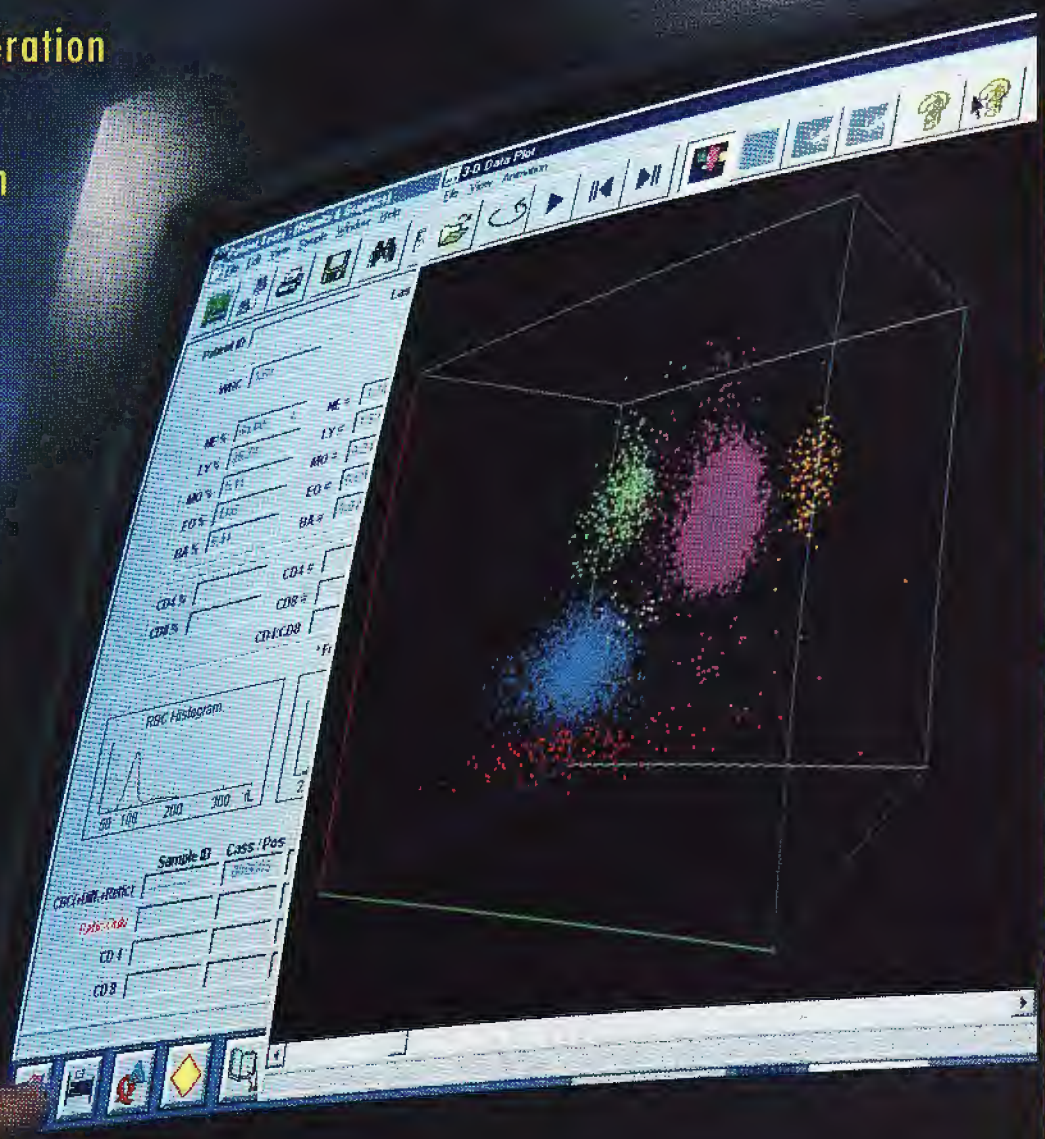

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