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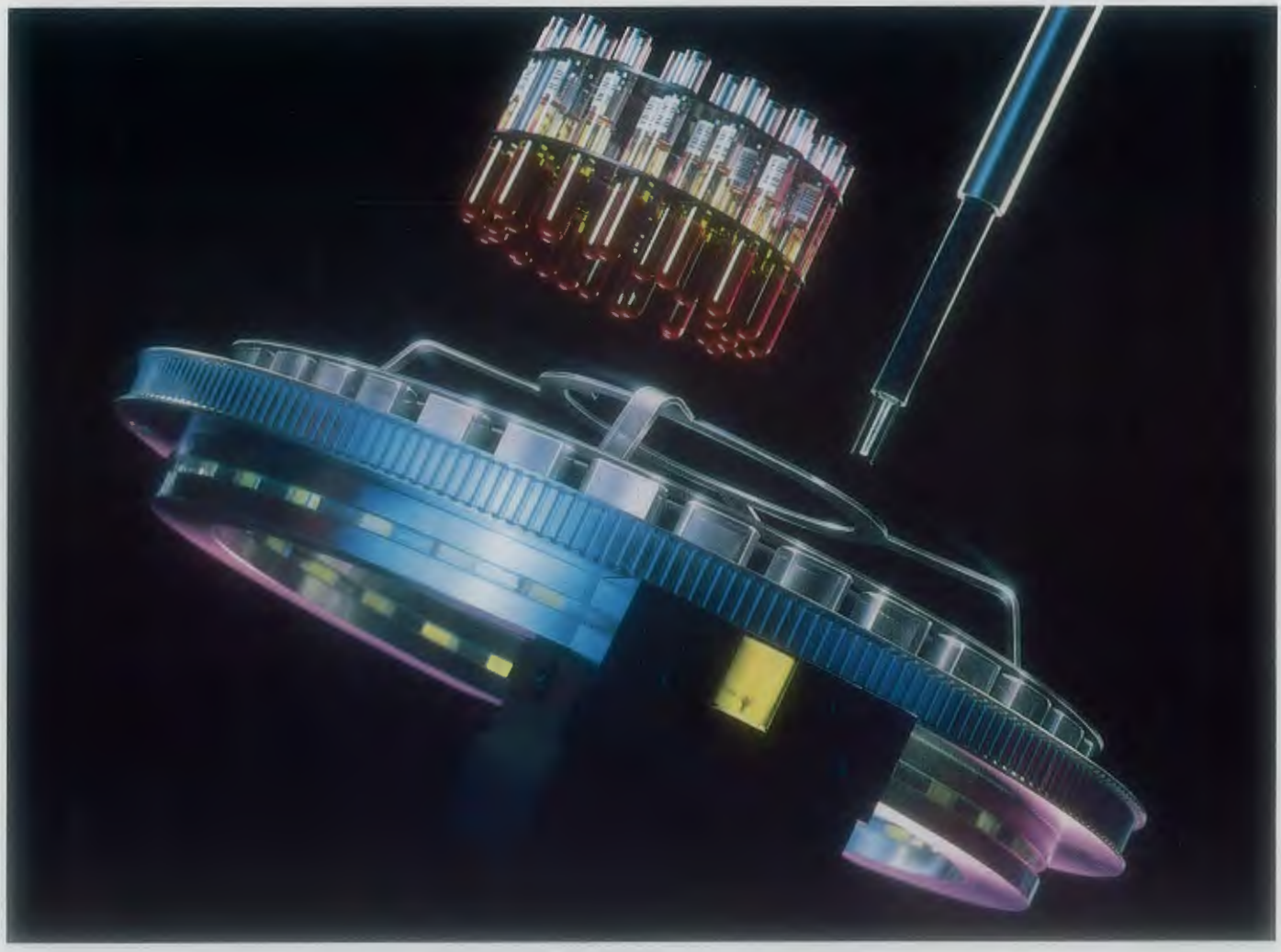

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Proliferating Cell Nuclear Antigen (PCNA) Expression in Archival Tissue. Evaluation of Microwave Pretreatment.

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Abstract
The effect of microwave pretreatment on antigen retrieval was assessed for proliferating cell nuclear antigen (PC10 clone) using lung tissue subjected to long-term 10% buffered formal saline fixation.

Evaluation of three microwave pretreatment protocols showed incubation in either 1% zinc sulphate or saturated lead thiocyanate to produce minimal background staining. PCNA indices from tissues pretreated in 1% zinc sulphate showed the closest correlation to cell proliferation kinetics determined by Ki-67 (MIB 1) staining. Pretreatment in 10 mmol citrate buffer resulted in excessive background staining and nuclear over-expression of PCNA.

Microwave pretreatment in 1% zinc sulphate is recommended as the method of choice for immunohistochemical localisation of PCNA following long-term formaldehyde fixation of tissues.

Key words
Proliferating cell nuclear antigen, Archival tissue, Antigen retrieval, Proliferation kinetics.

Introduction
Long term storage of tissue in formaldehyde-based fixatives has a deleterious effect on immunohistochemical expression of antigens for a wide variety of commercial antibodies. This loss of antibody labelling is probably due to the development of cross-links between formalin molecules and antigen-related proteins, and is directly proportional to the fixation immersion interval[1].

Microwave pretreatment of tissues has been shown to enhance antigen-antibody labelling and various pretreatment protocols have been proposed[2,3]. The results obtained from microwave pretreatment show considerable variation and the optimum protocol differs between antibodies.

This study was undertaken to evaluate the effect of microwave pretreatment on the immunohistochemical expression of the cell proliferation marker, proliferating cell nuclear antigen (PCNA) and to determine which pretreatment protocol produces optimum antigen labelling.

Materials and methods
Blocks of formalin-fixed paraffin-embedded lung tissue obtained at post-mortem examination of nine asthmatics were used in this study. Prior to embedding in paraffin, the tissue had been stored in 10% buffered formal saline for at least six weeks. Fresh sections were cut from each block and the blocks whose sections contained epithelial-lined proximal airways were selected for further study.

Serial sections, 4μ in thickness, were cut from each of the selected blocks and were mounted on poly-L-lysine coated slides. No external heat was employed to promote adherence of the tissue sections onto the slides. Four of the sections from each case were stained for PCNA using the strept-avidin biotin method. Three of these underwent microwave pretreatment in one of three incubation solutions: 1% zinc sulphate in distilled water, saturated lead thiocyanate in distilled water or 10mmol citrate buffer. The sections were incubated in the solutions in a microwave oven (Goldstar model ER-686JE, 700 Watts) at high power for five minutes. The solution was permitted to cool for one minute and was topped up as necessary. Following cooling the sections were incubated for a further five minutes at high power. The sections were then left to stand in the incubating solution for 15-20 minutes. In addition to those pretreated by microwave incubation, further sections from each of the caes were stained for PCNA using the strept-avidin biotin method without any microwave pretreatment.

Non-specific antibody binding was blocked using 0.5% casein followed by incubation overnight in PCNA PC10 clone (Dako product No. M879) diluted 1:100 in TRIS buffered saline. Following this sections were incubated in biotinylated rabbit anti-mouse antibody diluted 1:300 in TRIS buffered saline for 30 minutes. Sections were then washed in TRIS buffered saline and incubated for 30 minutes in strept-avidin biotin complex. After washing, the reaction was visualised with 3,3' diaminobenzidine. Haematoxyin was used as a counterstain and the sections were dehydrated, cleared and mounted in synthetic mountant.

A further section from each block, cut at 4μm thickness was stained for Ki-67, MIB 1 antibody (Dako product No. A047) using the strept-avidin biotin method as previously described (3).

Negative controls in which non-immune serum was substituted for the primary antibody were employed for each section examined. Sections of tonsillar tissue were used as positive controls for each batch of sections stained. The staining of bronchial epithelial cells obtained from each of the methods employed was assessed in a subjective manner with sharpness and specificity of nuclear staining, and the degree of non-specific background staining being recorded. In addition a proliferation index was devised for each section by counting the PCNA/PC10 or Ki-67/MIB1 positive nuclei present. The proliferation index was expressed as the percentage of labelled nuclei present with all bronchial epithelium in each section being assessed. An eyepiece integration grid (Zeiss integrationsplatte 1, integration grid, product No. 474140) was used to ensure that nuclei were evaluated once only.

Results
All sections studied showed some degree of nuclear-specific labelling following immunohistochemical staining with either PCNA/PC10 or Ki-67/MIB 1 antibody.

In those sections that underwent staining without microwave pretreatment, the nuclear staining was pale and difficulty was experienced in determining which nuclei showed PCNA positivity. Sections that were pretreated in citrate buffer showed marked overstaining with non-specific staining of epithelial cell cytoplasm and adjacent connective tissue. Those sections pretreated in citrate buffer showed marked overstaining with non-specific staining of epithelial cell cytoplasm and adjacent connective tissue. Those sections pretreated in zinc sulphate and lead thiocyanate all showed nuclear specific staining with minimal or no background staining. Nuclear staining was clearest in those sections pretreated with zinc sulphate and in this group there

was least difficulty in determining which nuclei showed positive labelling for PCNA.

The mean PCNA index and range of indices derived for each staining protocol and the Ki-67 index determined from the same area of tissue are shown in Table 1.

Comparison of PCNA indices obtained using each of the four pretreatment protocols with Ki-67 indices of sections from the same tissue blocks showed PCNA indices of the zinc sulphate pretreatment group to have the strong correlation (Pearson's correlation coefficient: no pretreatment, 0.134; cirate buffer, 0.066; zinc sulphate, 0.854; lead thiocyanate, 0.549).

Table 1

Asthmatic bronchial epithelium.

Staining protocol	Mean labelling index % (standard deviation)	Range of indices %
PCNA/PC10		
no microwave pretreatment	2.6 (2.9)	0.1-7.2
citrate buffer	47.7 (15.5)	27.6-72.4
zinc sulphate	6.0 (1.4)	4.1-9.2
lead thiocyanate	6.2 (4.3)	0.3-13.3
Ki-67/MIB 1	3.9 (1.1)	2.1-6.1

PCNA proliferation indices according to staining protocol and Ki-67 indices.

Discussion

PCNA is a cycling-cell specific antigen which shows maximal expression in the S-phase of the cell cycle[4]. In various series PCNA labelling has been utilised to detect the proliferative compartment in benign tissues[5,6] and has been correlated with various other markers of cell proliferation in malignant tissues[9,10].

Application of PCNA immunohistochemistry in archival tissues is hampered by variability of antigen labelling in tissues that have been stores for prolonged periods in formaldehyde-based fixatives[1,2]. This variability in staining results from the development of intermolecular cross-links that mask various antigenic epitopes[1,2].

The use of microwave processing to expose antigenic sites was initially advocated by Shi et al (1991) who demonstrated enhanced staining with a large variety of antibodies[2]. As part of their protocol they employed post-fixation in heavy metal based incubation solutions and suggested that the salts acted as protein precipitants which resulted in better preservation of antigens. In that study incubation in lead solution was found to result in slightly less background staining than was noted when zinc based solutions were employed, although varying results were obtained with differing antibodies.

Citrate buffer was found to produce optimal results in a series of studies in which MIB 1-3 antibodies were employed to detect Ki-67 antigen in routine histological material that had been embedded in paraffin following formalin fixation[3]. In subsequent studies incubation in citrate buffer has also been applied in the immunohistochemical analysis of various human tumours using a variety of antibodies[13,14]. In these later studies variability in staining was reported and it has been noted that the effect of microwave pretreatment on antigen expression in fixed tissue is dependent on the antigen which is being studied[13].

The results of the present study have confirmed that prolonged formalin-fixation results in reduction of PCNA expression when standard immunohistochemical staining techniques are employed. The application of microwave pretreatment has resulted in increased labelling of PCNA but the results showed considerable variation which was dependent on the nature of microwave incubation solution used. Of the three incubation solutions evaluated citrate buffer was associated with the most unsatisfactory results due to excessive staining. In tissues treated with citrate buffer the degree of non-specific staining resulted in PCNA indices that were far in excess of those derived from other methods and appeared unrelated to the true proliferative activity of the tissues under examination.

Both zinc sulphate and lead thiocyanate solutions produced minimal background staining although sporadic high PCNA indices and the wide range of scores obtained from lead thiocyanate treated tissue would suggest some degree of lack of specificity in staining.

When PCNA indices, derived using each of the protocols employed, were compared with proliferation indices from Ki-67/MIB 1 labelled bronchial epithelium the strongest correlation was derived from those sections pretreated with zinc sulphate.

MIB 1 is a formalin-resistant antibody whose expression shows close correlation with nuclear proliferation and with Ki-67 expression in unfixed tissues[3]. Comparison of MIB 1 and PC10 staining in proliferating hepatocytes and tonsillar tissue indicates that MIB 1 is expressed in a more confined segment of the cell cycle[5,6] and as such, MIB 1 indices would be expected to be lower than those observed for PC10. The higher PCNA/PC10 indices derived from citrate buffer and lead thiocyanate treated tissues therefore provides evidence of non-specificity of staining and suggest that these two methods produce unsatisfactory results for PCNA/PC10.

Conclusion

This study has shown that PCNA/PC10 staining may be enhanced by microwave pretreatment of sections. Incubation of tissues in 10% zinc sulphate solution produced satisfactory staining that appeared to reflect the proliferative activity of the tissue examined.

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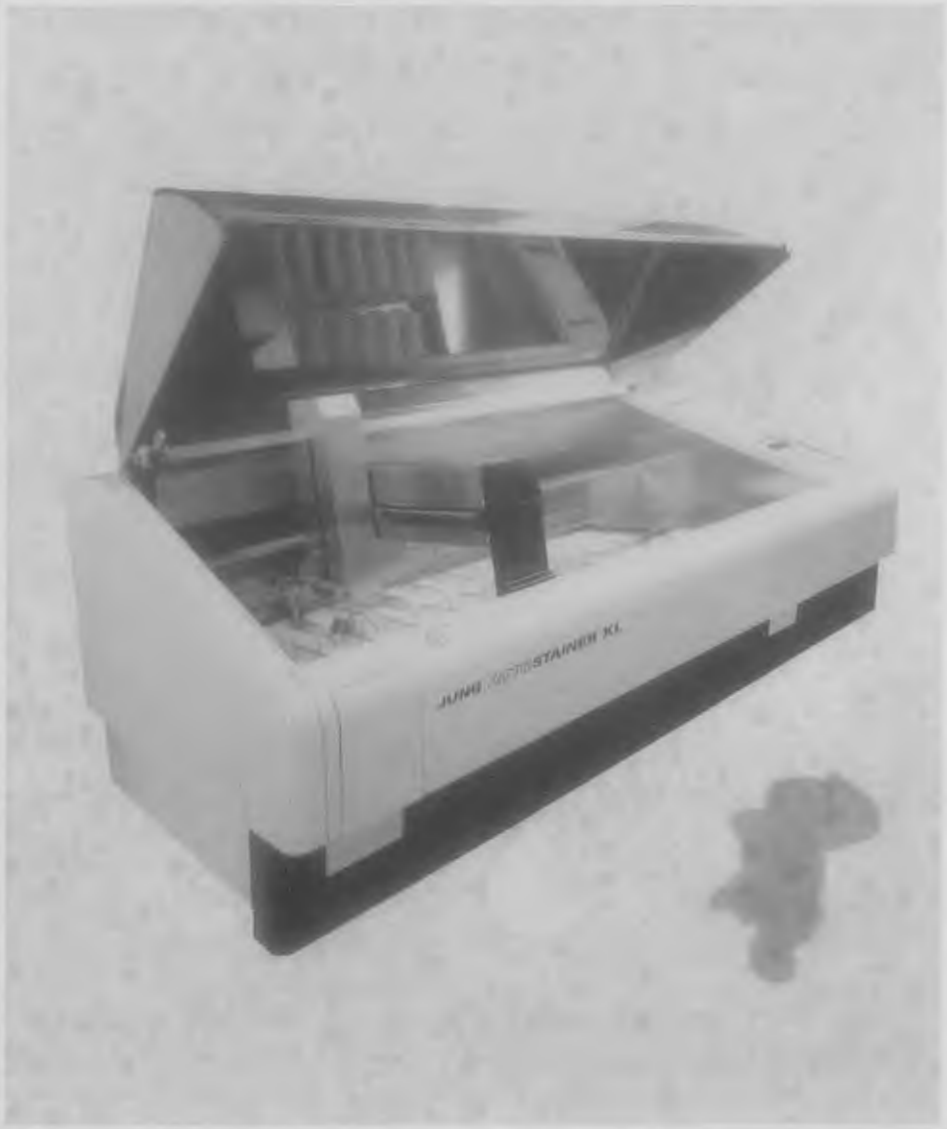

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Caffeine as an Accelerator of Bilirubin Extraction from Amniotic Fluid

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ABSTRACT

A method of extraction of bilirubin from amniotic fluid into chloroform was developed with caffeine as accelerator. It requires 2 mL of specimen, mixing for 10 minutes with chloroform containing 100 g of caffeine per L and reading the absorbance of the organic phase at 453 nm. After using 520 nm as a secondary wavelength to limit nonspecific interference, it had a coefficient of variation of 0.018 (1.8%) at an absorbance of 0.066, showed negligible interference from oxyhaemoglobin, methaemoglobin, haematin and biliverdin, and was linear to an absorbance of at least 0.6. Although addition of TES buffer (pH 7.0, final concentration 0.1 mol/L) increased recovery of exogenous bilirubin added to amniotic fluid, it was without significant effect in most routine specimens. Extraction with chloroform-caffeine showed results similar to those from direct spectrophotometry but without spectral interference. Compared with other extraction methods, it offers comparable efficacy while avoiding use of aniline and difficult separation of the phases.

Key words:

bilirubin, amniotic fluid, erythroblastosis, fetal

Introduction

Examination of amniotic fluid in the management of pregnancies complicated by rhesus sensitisation was first proposed more than 40 years ago[1]. The major objective of testing was to distinguish the mildly or unaffected fetus from the fetus with severe disease[2]. The fetus only mildly affected could then remain in utero to gain maturity while the more severely affected fetus could be monitored carefully to achieve the best compromise between prematurity and anaemia. In the ensuing years, monitoring by ultrasound has diminished the importance of chemical analysis of amniotic fluid in assessing fetal well-being, and other interventions have become possible through the use of intrauterine transfusion and fetoscopy. Nevertheless, amniotic fluid analysis retains a role in fetal monitoring, especially so in country areas lacking either ultrasound equipment or expertise in its use.

Liley[3] related the absorbance of amniotic fluid at 450 nm to the presence of haemolytic anaemia, and provided guidance for the clinical management of rhesus-sensitised pregnancies. His measurements at 450 nm, presumably representing concentrations of bilirubin, were corrected for the contribution of oxyhaemoglobin and of non-specific background interference by plotting the absorbance at more than 20 fixed wavelengths on a logarithmic scale against wavelength. We have used Liley's procedure routinely but resort to extraction of amniotic fluid with chloroform-aniline[4] whenever gross interference invalidates direct spectrophotometry.

We find both of these methods cumbersome in routine use. Liley's original method requires both careful plotting of a large number of absorbance values and some experience in interpreting the extent of correction. Failure to observe eithre requirement can produce clinically misleading results. Extraction with chloroform-aniline frequently results in an organic phase contaminated with proteinaceous material that is difficult to clear.

In view of these problems, I investigated alternative procedures. Manual plotting might be superseded by use of a scanning instrument but even the most sophisticated data handling cannot overcome interference from other pigments that the Liley method is known to suffer[5]. As a centre receiving specimens from country areas, we find contamination a continuing problem whether or not amniocentesis has been guided by ultrasound. Indeed, a recent comparison of methods suggested that contamination by blood may be greater than generally appreciated[6].

A method exploiting photodegradation of bilirubin seems unaffected by contaminating pigments[7], but it does not appear to be widely used, possibly because its underlying principle is unusual in the routine laboratory. Instead of exploring this approach, I therefore elected to examine further an extraction procedure as we have experience of this technique. I found that addition of caffeine to chloroform promoted extraction of bilirubin into the organic phase, providing a method for amniotic fluid that was quick, simple and robust in routine use.

MATERIALS AND METHODS

Chloroform, caffeine, aniline and dimethylsulphoxide were all of Analar grade from BDH Chemicals, Palmerston North, New Zealand. MES (2-(N-morpholino)ethanesulphonic acid), TES [N-tris(hydroxy-methyl]-methyl-2-aminoethanesulphonic acid) and tris (tris(hydroxy-methyl)aminomethane) were from Sigma Chemical Co., St. Louis, MO, USA. Human albumin obtained in solution (200 g/L) from Commonwealth Serum Laboratories, Melbourne, Australia, was diluted with physiological saline (0.15 mol/L) before use. Bilirubin from Calbiochem, San Diego, CA, USA, was dissolved in dimethylsulphoxide before addition to album solution, giving a dimethylsulphoxide concentration finally of no more than 0.3%. Oxyhaemoglobin was prepared in crude form from a specimen of heparinized whole blood by washing the red cells twice with physiological saline, making up to the original volume with water and standing overnight. After removing any unlysed cells and cellular debris by centrifuging at about 1000 g for 10 mins at 4°C, the supernatant layer was used as a stock haemoglobin solution. Methaemoglobin was prepared by treating 5 vol of stock haemoglobin solution with 1 vol of $K_3Fe(CN)_6$ (200 g/L). Haematin hydrochloride (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in NaOH solution (0.1 mol/L) to give a stock solution containing 2 g of haematin/L. Biliverdin was prepared in crude form by adding 1 vol of $FeCl_3$ solution (about 3.7 mol/L) to 100 vol of bilirubin solution (1 g of bilirubin/L of dimethylsulphoxide) and standing at room temperature for 2 hours. Spectrophotometry was carried out by using a Uvikon 860 spectrophotometer (Kontron AG, Zurich, Switzerland). Least-squares linear regression analysis of method comparison data was done by Deming's method (8). Other techniques of method evaluation were based on those described elsewhere[9].

The final procedure adopted was as follows. Amniotic fluid was centrifuged at about 1000 g for 10 min at 4°C to remove contaminating cells. Any red cells found at this stage were retained for detection of fetal haemoglobin by Kleihauer test. Two mL of supernatant fluid were transferred to a glass tube (approx 10 x 1 cm with a ground glass stopper) and mixed with 0.1 mL of TES buffer (2 mol/L, pH 7.0). Two mL of chloroform containing caffeine (100 g/L) were added, and the tube stoppered securely before mixing by inversion on a rotary mixer (approx 30 rpm) for 10 min. After separating the phases by centrifuging at about 1000 g for 5 min at 4°C, about 1 mL of the lower, organic phase was pipetted into a glass semi-micro cuvette and its absorbance read at 453 and 520 nm against air. The spectrophotometer automatically calculated the absorbance difference (453-520 nm) which was reported directly.

RESULTS AND DISCUSSION

Factors affecting extraction. When using bilirubin (about 10 μmol/L) in albumin solution (40 g/L) as a model system, factors affecting extraction of bilirubin into chloroform were (i) the presence of additives in the organic phase and (ii) vigour of mixing. I confirmed that addition of aniline to chloroform (to give 1% v/v) increased extraction of bilirubin into the organic phase (4), but pretreatment of the specimen with buffer of pH 8.15[14] was without additional benefit. Addition of caffeine to chloroform either with or without aniline promoted further extraction of bilirubin (Fig. 1) with a progressive loss of effect of aniline at the higher concentrations of caffeine. Caffeine was without effect on the spectral properties of bilirubin in the organic phase: the peak remained at 453 nm and the absorbance at the peak was unchanged. Subsequent experiments were done with 100 g of caffeine/L of organic phase.

More vigorous mixing, either by shaking (instead of simply inverting) the tubes or by using smaller volumes (2 instead of 3 mL of each) of amniotic fluid and organic phase, gave increased extraction at the expense of more difficult separation of the phases. To ensure uniformity of mixing, I substituted a rotary mixer for manual mixing. Under these conditions, 2 mL volumes gave both clear separation of the phases and sufficient material for spectrophotometry with a semi-micro cuvette whereupon a true accelerating effect of caffeine in comparison with chloroform-anline alone was observed (Fig 2).

Interference. To limit the effect of light scattering by droplets of water that occasionally contaminated the organic phase, I used a secondary wavelength of 520 nm where bilirubin does not absorb. Deliberately contaminating the organic phase by progressive remixing with aqueous phase caused absorbance at 453 nm to increase from 0.299 to 0.340 to 0.382 whereas for 453-520 nm the increase from 0.303 to 0.315 to 0.322 was less marked.

Interference from other pigments was investigated by extracting albumin solutions containing respectively haemoglobin (1.3 g/L), methaemoglobin (1.1 g/L), haematin (0.02 g/L), and biliverdin (about 15 μmol/L). For the first three pigments, the organic phase had very low absorbance (0.002), no different from that after extracting albumin solution alone. Interference from biliverdin was less straightforward to assess in that the organic phase contained significant quantities of yellow material absorbing at 453 nm while the aqueous phase remained green. Spectral analysis of the organic phase revealed its absorbance characteristics to be identifical with those of bilirubin (broad peak at about 450 nm) and quite unlike those of the crude biliverdin solution added directly to the organic phase (peak at about 360 nm with a small shoulder at 453 nm). The likelihood is that this material was bilirubin that had failed to be oxidised; in any event, based on the absorbance of the extracted material at 360 nm, the contribution from biliverdin at 453 nm was insignificant (less than 1% of that arising from the contaminating pigment).

Linearity. Extraction was linear ($1 - r^2 < 10^{-4}$ for all data) to an absorbance difference of 0.6 (equivalent to a bilirubin concentration of about 10 μmol/L), the highest value examined, with identical results in the presence and absence of aniline (Fig. 3). For the data without aniline, the intercept was 0.0016 absorbance units, slope 0.058 absorbance units. L μmol^{-1} and SEE 0.0005 absorbance units.

Omission of aniline. The observations presented on concentration-dependence, on time-course and on linearity (Figs. 1-3) showed no additional effect on aniline when caffeine was present at a concentration of 100 g/L. Omission of aniline could therefore be instituted without prejudice to the efficacy of extraction. Moreover, in subsequent experiments, omission of aniline enabled spectrophotometry to be done without a solvent blank: absorbance at 453-520 nm with air as blank was from an analytical standpoint insignificantly higher than that with a solvent blank (mean ±SEM =

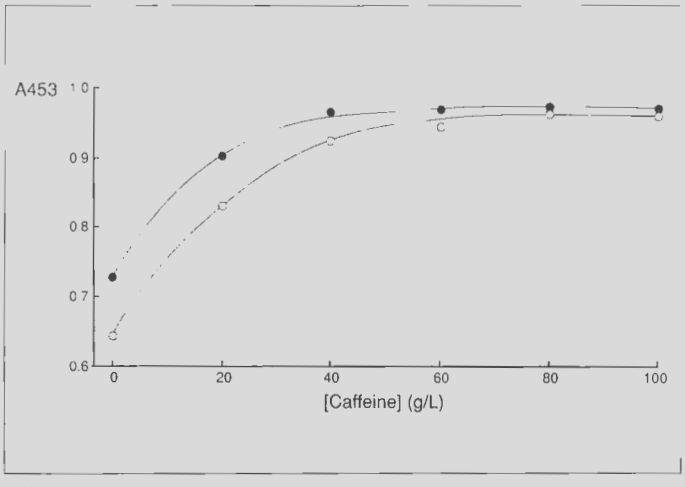

Figure 1.
Effect of caffeine on bilirubin extraction.
Caffeine was dissolved in chloroform with (●) or without (O) aniline (1%) to give the concentrations shown, and the organic mixtures were shaken with saline solution containing albumin and bilirubin. The absorbance is of the organic phase after centrifuging.

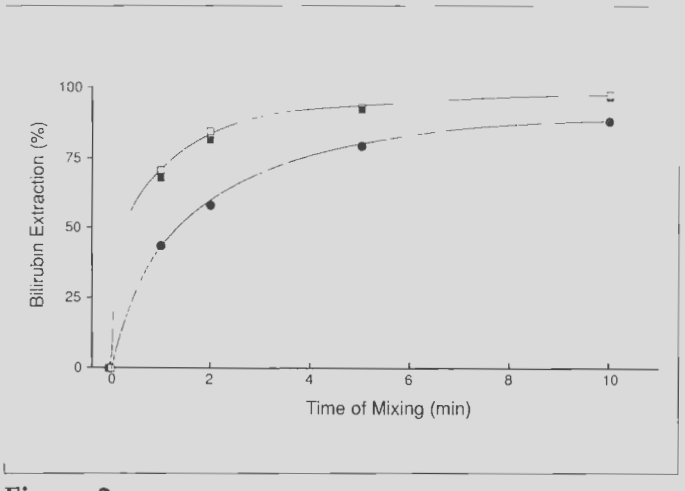

Figure 2.
Effect of time of mixing on bilirubin extraction.
Chloroform containing aniline (1%) only (●), caffeine (100 g/L) (□) only, or aniline plus caffeine (■) was mixed with saline solution containing albumin and bilirubin for the times indicated. After centrifuging, the absorbance of the organic phase was expressed as a percentage of that of bilirubin added directly to organic solvent.

0.0031 ± 0.0003 absorbance units for 12 observations on amniotic fluid specimens).

Imprecision of the procedure was assessed by analysis of 30 amniotic fluid specimens giving absorbance values of 0.009-0.223. Duplicate determinations had a mean SD of 0.0012 (difference between duplicates ranging from 0-0.005) absorbance units representing a coefficient of variation of 0.018 (1.8%) at the mean value of 0.066. The method was therefore sufficiently precise to allow singleton assay to be done routinely.

Recovery. Because of the difficulty of obtaining sufficient material on a large number of specimens, recovery experiments were done with pools of stored amniotic fluid, each pool from a small number of patients. On extraction, the base material had nil absorbance at 453-520 nm. In 8 separate pools, addition of bilirubin sufficient to give an absorbance of about 0.25 when added directly to chloroform-caffeine resulted in recoveries of 68-86 (mean ± SEM = 77 ± 2)%. These recoveries seemed lower than when albumin solution was used (e.g. see Fig 2). To test whether change of pH (possibly occurring on storage) might be a causative factor in lowering recovery, bilirubin was extracted from albumin solution made up in physiological saline, in borate buffer (0.1 mol/L, pH 9.2), and in succinate buffer (0.1 mol/L, pH 4.0). The recoveries were respectively 91, 30 and 7%. These preliminary observations were extended in a more systematic study (Table) indicating that pH 7 was optimal for bilirubin extraction. After finding that the amniotic fluid pools had pH values of 8.3-8.7 and adjusting their pH and TES buffer (pH 7.0, final concentration 0.1 mol/L) to between 7.00 and 7.05, recoveries in the same 8 pools were 80-93 (mean ± SEM = 86 ± 2)%. A second extraction of the aqueous phase gave trivial increments equivalent to no more than 5%, indicating that a single extraction was adequate and suggesting that the recoveries were falsely underestimated.

Method comparison. In 35 amniotic fluid specimens of chloroform-caffeine extraction with direct spectrophotometry as described by Liley[3] gave the regression equation: extraction absorbance = 0.975 x direct absorbance - 0.001; r = 0.983 and SEE = 0.013 absorbance units. A difference plot (10) (Fig 4) suggested no constant difference, with smaller percentage differences at higher absorbances, albeit with limited data. Using the criteria of Liley[3]

Table 1

ADDITION	RECOVERY (%)
MES (0.5 mol/L, pH 6.0)	70
MES (0.5 mol/L, pH 6.5)	95
TES (2 mol/L, pH 7.0)	96
TES (2 mol/L, pH 7.5)	94
Tris (2 mol/L, pH 8.0)	90

The additions indicated were added as 100 µL aliquots to 2 mL of saline containing albumin and bilirubin. Each specimen was mixed with chloroform-caffeine as described in the text, and the absorbance of the organic phase expressed as a proportion of an equivalent concentration of bilirubin added directly to chloroform-caffeine and giving an absorbance difference of 0.431 at 453-520 nm. The proportions shown are means of duplicate determinations.

throughout, the disparities between the results from the two methods would not have led to differences in interpretation. It is worth noting, however, that one very large discrepancy (78%, not shown in Fig 4) resulted from miscalculation of the absorbance data (Liley method) and led to unwarranted clinical intervention. Timely use of the extraction procedure in this case would have avoided such mismanagement.

Effect of buffer. Comparison in 16 specimens of the effect of TES buffer on extraction showed a small increment with buffer of 0.004 + 0.0007 (SEM) absorbance units which was statistically ($p < 0.001$) but not clinically significant. However, one additional specimen showed an increase with TES of 0.052 absorbance units, a discrepancy of 86%; although I was unable to confirm this result because of insufficient specimen, the possibility that such a large error might arise in the absence of buffer argues for continued use of pH adjustment.

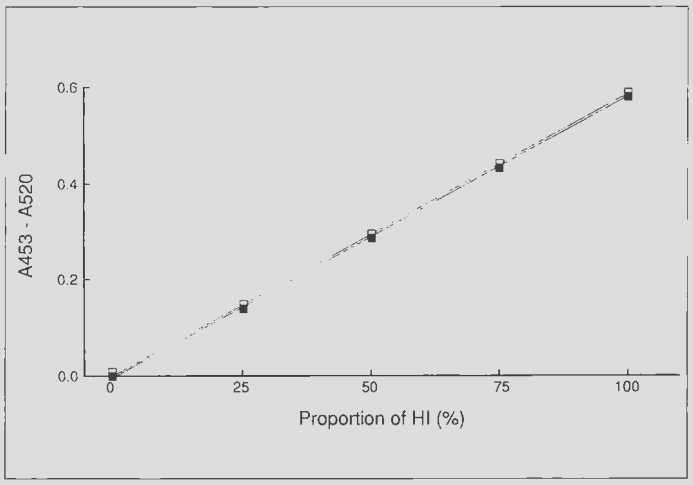

Figure 3.
Linearity.
Saline solution containing albumin and bilirubin ("HI") was pre-mixed in various proportions with an identical solution lacking exogenous bilirubin, and mixed for 10 minutes with chloroform containing caffeine (100 g/L) either with (■) or without (□) aniline (1%). Thje absorbance is of the organic phase after centrifuging.

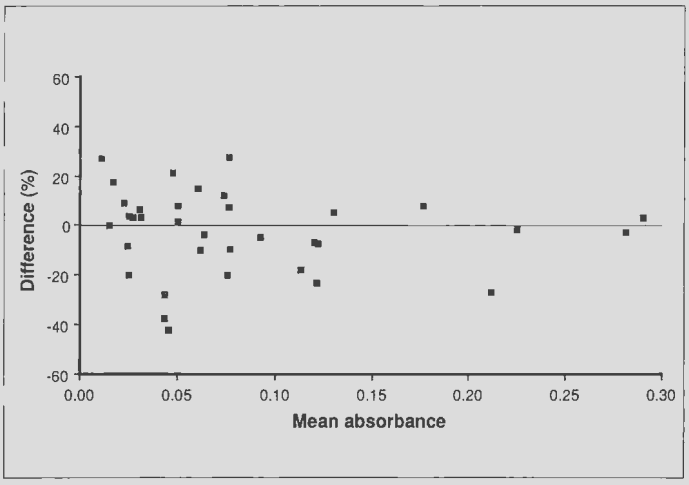

Figure 4.
Difference plot for chloroform-caffeine extraction and Liley methods.
After calculating the mean ([extraction + Liley]/2) absorbance for 35 amniotic fluid specimens, the difference between the methods (as [extraction - Liley]/mean) was plotted as a percentage.

Conclusions

The procedure described in this paper offers an extraction method for amniotic fluid analysis that is mild enough to avoid problems with protein denaturation while achieving effective transfer of bilirubin to the organic phase. The use of caffeine seems to be a novel approach but one that is immediately suggested by caffeine's application as an "accelerator" in total bilirubin methods[11]. The technique is precise, more so than those described by others[5], enabling a reliable result to be given by extraction of a single 2 mL specimen. It is free from interference due to haemoglobin and related pigments, and does not use aniline, a toxic substance. In comparison with Liley's original method, the extraction procedure is unaffected by interference (cf. ref 5) and avoids a protracted calculation that may induce error sufficient to invite clinical mismanagement. The mean values obtained are so similar to those of Liley's method that his interpretative charts[3,12] can be used directly.

Abbreviation:

The abbreviations used are MES, 2-(N-morpholino)ethanesulphonic acid, TES, N-tris (hydroxy-methyl)-methyl- 2-aminoethanesulphonic acid and tris, tris (hydroxymethyl)aminomethane.

Ethics:

The procedures followed were in accordance with the ethical standards set by the Auckland Area Health Board Ethics Committee.

Acknowledgment:

I thank Drs JR Baker and AB Roberts for their helpful comments on the manuscript.

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How Will The "New" Health System Serve its Customers? – a Maori focus

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The New Zealand Health Service has a responsibility to deliver quality health care to all New Zealanders in the most effective and cost-efficient way, since just as all New Zealanders are potential customers of the Health Service, so are they also the source of funding for the service through their tax payments. Thus as customers they will want the best service, but as funders they will want the money spent carefully. All New Zealanders are customers, but the delivery of health care to the Maori customers is of particular relevance if commitment to honouring the principles of the Treaty of Waitangi is to be of more than a superficial nature.

The Treaty of Waitangi and its Implications for the Maori Health Issue

Maori health is of particular concern to all New Zealanders as it has now been related by Maori to the Treaty of Waitangi; in his book "Hauora – Maori Standards of Health" pp 20-21[1], Professor Eru Pomare states that:

"For Maori people the Treaty articulates their status as Tangata Whenua [indigenous people]; guarantees their rights with respect to land, water, forests, fisheries *and other treasures*, and confirms their rights to Mana Motuhake [self-determination]. The signing of the Treaty by both Maori and Pakeha was seen as the recognition of a partnership of equals and the basis of the relationships between the two races. Implicit within the Treaty were the concepts of equity, partnership, and economic and cultural security, all of which contributed importantly to Hauora (spirit of life/health). Poor standards of Maori health may therefore be regarded in part as non-fulfilment of these Treaty concepts and obligations."

These "other treasures", or taonga, are taken to include this right to good health. Of course it is not possible to separate completely all of the other economic, land and social issues from health, so any current areas of conflict over the honouring of the Treaty are seen as having an impact on health.

The New Zealand Health Service has taken this proposition seriously, as is shown in a Department of Health Circular Memorandum [2] issued in 1988. This memorandum was to bring to the attention of the Health Service, the recent Government discussion paper on "Partnership Perspectives"[3], and show how this was related to health. The folder accompanying the memorandum contained resource material including the Government Acts relating to the Treaty, [4,5] a study of the Treaty by Manuka Henare and Edward Douglas [6], and the Royal Commission on Social Policy's discussion booklet on "The Treaty of Waitangi and Social Policy"[7], and an editorial (source not given) called "Toward Multiculturalism" by Margaret Ramsay [8], which related the bicultural process to multiculturalism. All of this material provided an excellent range of fact and opinion to educate health professionals in their responsibilities under the Treaty towards Maori health care. One point to consider in all of this, however, is how widely this information was desseminated, and how far down the "chain of command" this type of material trickles.

The memorandum itself[2] identifies the two key issues of the Treaty with particular implications in the health are as *partnership* and *the protection of taonga (treasures).*

Under the partnership heading are itemised the particular ways that partnership could be implemented by involving Maori in:

* all levels of policy making and administration
* drafting, implementing and monitoring of legislation
* decisions on suitable social services

The statement of Professor Mason Durie, a member of the Board of Health and the Royal Commission on Social Policy was quoted, in which he explained the relationship between taonga and Maori health status. He drew the conclusion that in so far as the Treaty has not been used to protect the taonga of land, family integrity and language, the health of Maori people has suffered. It was affirmed that the Department of Health recognised that concepts of health for Maori people are firmly based in the Maori culture, and that Maori have the right to appropriate services funded through the health service.

There then follows a response from the Department of Health on ways that this situation can be addressed. It is noted that in "Partnership Perspectives"[3], it was stated that mainstream departments are not responsive enough to the needs of Maori people and communities because they are predominantly monocultural in outlook and personnel. It then lists initiatives which have been taken, such as the development of a departmental kaupapa (culture), appointing senior Maori advisory staff, devolving responsibility for Maori health, and training staff in the importance of bicultural issues.

Health education in New Zealand has also taken this responsibility seriously, with virtually all training programmes for health professional now containing section/s which deal with informing their students, who are the future health workers, on the significance of the Treaty. As shall be discussed later, many health providers have now instituted programmes to try to fulfil this responsibility.

Maori View of Health

Rose Pere, in "He Matapuna" [9], an essay on Maoriness, recognises five essential dimensions that influence her being and her approach to life. These are:

* Spirituality (wairua)
* Ancestral ties (whakapapa)
* Kinship ties (whanau)
* Humanity as a whole
* Earth (Papatuanuku) as part of a vast universe

These relate closely to the Maori view of health, which traditionally sees it as being an all-embracing concept which emphasises four important aspects (1 p.22):

* Wairau (spirituality)
* Whanau (family)
* Hinengaro (mental)
* Tinana (physical)

While some non-Maori might say that this is not a solely Maori viewpoint, it is important to recognise that a commitment to the health of our Maori customers must also involve a commitment to these values. It would be hoped of course that the benefit would spill over

onto all of the customers of the Health Service; this in fact is one of the pluses of acknowledging biculturalism, in that the good features of Maori philosophy can be used for the advantage of all.

The State of Maori Health – Why is it an Issue?

If we are to be concerned about the delivery of health care to Maori as customers of the Health Service, it is useful to consider if there is a special case here, or are the problems of Maori Health simply the same as the problems of the rest of the customers?

In the 1993 New Zealand Official Yearbook p.79 [10], the population of New Zealand is given as 3.38 million in 1991, of whom 323,000 or 9.6% are Maori. It also recounts how the Maori population at the first time of European contact in 1769 was estimated between 100,000 and 200,000. Though estimates vary, it is agreed that during the next 70 years there was a rapid decline in the population numbers as the Maori were ravaged by the newly introduced European diseases such as tuberculosis, typhoid, venereal disease, measles and other childhood illnesses. Firearms and warfare also contributed to this drop in population, but by the time of the first census in 1858, numbers had dropped further to only 60,000, with the lowest point of 42,000 in 1896. Thus Maori health was a serious problem from the time of the first contact.

Professor Pomare's book "Hauora" [1] deals with 1970-1984, and builds on an earlier publication covering the 1950-1975 period. The statistics show a steady pattern of poorer health in most of the areas which are examined. Examples of this are:

* A greater percentage of Maori infants are of low birthweight. In 1984, 7.4% were below 2500g compared to 5% of non-Maori infants (p33).
* The cot death rate for Maori infants is on average nearly twice that of non-Maori infants, and is the main contributor to infant deaths (ibid).
* At ages 25-64 years, total death rates for Maori women are twice that of Maori women (ibid).
* Respiratory diseases cause 2-3 times more deaths in Maori people at all ages (p52).
* Maori infants continue to have a public hospital discharge rate 2 times higher than non-Maori infants, and the rate of acute respiratory infections is nearly 4 times higher than non-Maori (p85).
* Maori rates of admission to psychiatric hospitals for schizophrenic psychoses are twice the non-Maori rate for males and 2.8 times higher for females (pp115-8).

This pattern may be improving in some areas (see initiatives covered later), but the 1993 Yearbook (10), still shows a record of poorer health. Some of the most disturbing figures are seen in Table 7.4 on page 149 which documents the rate of children's hospital discharges. Overall there is about a two times greater rate for Maori as opposed to non-Maori, while the age 1-4 grouping has 335 per 1000 for Maori against 130 for non-Maori _ almost three to one.

The infant mortality statistics (p158-9), while showing a twofold decrease since 1960, still contain depressing details. While the late foetal to neonatal figures are only slightly worse than non-Maori, the post neonatal and infant figures are more than double the non-Maori rate. Looking at the tables which show the principal causes of these deaths, the most worrying ones are the rate of respiratory disese death per 1000 being 1.4 for Maori, and only 0.1 for non-Maori, sudden infant death syndrome at 7.6 as compared with 2.1, immaturity at 0.9 as opposed to 0.4, respiratory distress syndrome 1.3 versus 0.7, and accidents 0.4 versus 0.2.

Government Policy Initiatives and the Signifance of the Purchaser/Provider Split for Maori Health.

It is against this background that moves have been taken at a policy level by the Government in declaring its Maori Health objectives. The Ministerial Advisory Committee on Maori Health in 1990 published "Policies for Maori Health Guidelines for Area Health Boards" [11] in which the need for Maori Health policies was described. Suggestions were made for ways in which Maori could participate in the planning and delivery of health care at iwi, hapu, whanau and an individual level, as well as by participating on the committee and board structures of the existing health system.

Bill Birch, Health Minister (12) declared the Government's primary Maori health objective to be, to seek to improve Maori health status so that in future Maori will have the same opportunities to enjoy at least the same level of health as non-Maori. He recognised the urgency of this and asked that Maori be involved in identifying needs and contributing to resolving them. He said that the new health service would

"promote, encourage or ensure:

* the greater participation of Maori people at all levels
* resource allocation priorities which take account of Maori health needs and perspectives
* the development of appropriate practices and procedures as integral requirements in the purchase and provision of health services"

He saw the purchaser/provider split as being an important issue for Maori, since the RHAs are responsible for buying the best health services to meet the needs of their customers; Maori are thus in a position to demand the service they want for their people and participate in the provision of these. This is an area which Maori community leaders need to take a positive stand, and speak out loudly and firmly with their requests for recognition of the special needs of the Maori customer.

Maori health is also included in the Health and Disability Support Goals for 1994(13).

Health Provider and Maori Initiatives

Many positive actions are now being taken to try to serve better the Maori customers of the RHAs in the limited space available only a few of these can be covered. The important point is, that the Maori view of health is being considered seriously and Maori are identifying problem areas and instigating action.

In 1989 the Race Relations Office prepared a report for Auckland Hospital on ways to confront institutional racism within the hospital [14]. Out of this arose the setting up of **Te Whanau Atawhai** (the caring family), based in the Starship Hospital, but serving all of Auckland Hospital [15]. The purpose of this unit is to provide support to families/whanau, accommodation for those from out of town, cooking and laundry facilities, and generally to provide liaison between patients, family and health professionals. This is aimed at making Maori feel at home in the hospital environment, and by treating the whole person achieve better health results. While set up originally by and for Maori, the unit is open to people of all races and cultures.

Northland Health runs the **Ringa Atawhai** (carying hands) programme [16] which aims to help people who have missed out on conventional health services through cultural differences and discomfort with the health system. Members of **Ringa Atawhai** are chosen from within their own communities, and after training, work within these communities on problems such as drug and alcohol abuse, physical and sexual abuse, diabetes and asthma.

In 1990 a hui was held at Ratana Pa – Huia Hauora Mokopuna [17] to develop strategies to improve the health of Maori

children in the 0-5 age group. The major problems were identified, and for each of these a list of practical suggestions was made e.g., encouraging breastfeeding for at least the first six months of a baby's life, discouraging smoking, and writing health education material in Maori and making it available at Kohanga Reo.

The Kohanga Reo movement has organised a medical insurance scheme at a very attractive price of $20 per child per year (18).

The cervical screening programme organised by the Department of Health focuses particularly on Maori Women[19], and makes a point of the most appropriate ways to promote this within the Maori community. Culturally sensitive issues such as the womb being seen as the "whare tangata" or house of man are addressed.

Maori Heart Health[10] p.161, has developed projects such as a rheumatic fever pilot project and an anti-smoking campaign. The aim of these is to foster a change of attitude and lifestyle and increase the participant's life expectancy for the benefit of mokopuna.

The Maori Women's Welfare League has been active in a number of projects. They helped the Tamaki Makaurau Health Commitee[10] p.161, in South Auckland to run a successful immunisation programme, and run a mental health awareness programme. Other Maori women of the Te Puawai Tapu (the Sacred Blossoming) are working with the New Zealand Family Planning Association to meet the common goal of "children by choice and not by chance".

Conclusion

Current awareness of the important of settling the issues of the Treaty of Waitangi as far as land claims go should also focus on the responsibility that the partnership places on all New Zealanders in areas such as health as well. The Maori customers of our Health Service deserve at the very least a fair share of the health "pie", as well as a firm commitment to addressing the inequities which have accumulated over the 352 years since first European contact. If we are committed to delivering the best service to our customers, we should be pro-active in identifying any areas where either we, or the organisations we are part of, could be doing more to ensure delivery of high quality and appropriate Maori health care.

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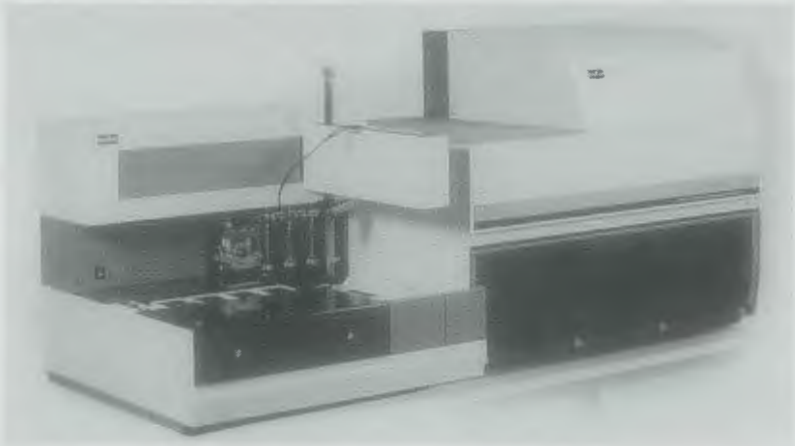

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.....

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BRIEF COMMUNICATION

The Use of the HemoCue in a Routine Haematology Laboratory

John W. Peters, MNZIMLS, Bernard C. Chambers, FIMLS, BSc.
Haematology Department, Middlemore Hospital,

Address for correspondence: John W. Peters., Haematology Dept., Middlemore Hospital, Private Bag 99311, Otahuhu, Auckland.

NZ J Med Lab Science 1994; 48 (3): 118-119

INTRODUCTION

Our laboratory has recently introduced the use of a HemoCue haemoglobinometer measuring device for establishing haemoglobin levels on selected patients.

The main reason for the purchase of such an instrument was that our existing Dade dilutor and Haemoglobinometer were unserviceable and to replace them would be far more expensive then the HemoCue.

The HemoCue was evaluated as an instrument that would take over the function of the Dade diluter and the Haemoglobinometer. The other major benefit that was envisaged was that because of the two wavelength technique of measuring the haemoglobin, the requirement to undertake the time consuming spun haemoglobin technique would be negated.

METHODS

The HemoCue comes from the HemoCue AB company in Sweden. The New Zealand agent is Biotek. The principle of haemoglobin measurement by the HemoCue is as follows:-

1. One drop of capillary blood/EDTA is placed in the self-filling microcuvette. The blood combines with the dry reagent (sodium desoxycholate, sodium nitrite, sodium azide and sodium fluorosceine). The sodium desoxycholate haemolyses the erythrocytes and haemoglobin is released. Sodium nitrite converts haemoglobin to methaemoglobin, which together with sodium azide gives azidemethaemoglobin.
2. After the introduction of the cuvette into the instrument the measurement is made at two wavelengths, namely 570nm and 880nm. This ensures automatic compensation for turbidity (lipaemia and increased WBC).
3. The results are available on the LCD screen in less than 60 seconds.
4. The instrument comes complete with a control cuvette that we use prior to each batch of tests that we run. We also run frequent checks between samples that have been run on the Coulter STKS.

The evaluation that was undertaken was quite brief as the Dade dilutor and Haemoglobinometer that we were using for these haemoglobin estimations died a short time into the evaluation.

The Coulter STKS haemoglobin measurement involves the addition of lysing agent to a dilution of EDTA anticoagulated blood the subsequent measurement of light transmitted through a filter of wavelength, 525nm, and on to a photocell. The photocurrent thus generated is proportional to the haemoglobin concentration.

The spun haemoglobin method involves two techniques, one for lipaemic samples and one for high white cell count samples.

a. Lipaemic samples:- An aliquot of EDTA sample is spun down at 3000RPM for 5 minutes and the top of the plasma level is marked on the tube. The plasma is removed down to the top of the red cell level and then the plasma is replaced by isoton. The sample is then mixed and re-sampled through the Coulter STKS.

b. High white cell count samples:- The standard dilution for a manual haemoglobin as done for the Coulter haemoglobinometer is done and the lysing agent is added. The sample is then spun at 3000RPM for 5 minutes and the haemoglobin is read in the haemoglobinometer.

We did have prior information regarding the correlation of the HemoCue in normal patients from work done at Norfolk Island Hospital, Red Cross Blood Transfusion Service, Sydney and the Auckland Regional Blood Centre. An evaluation of the HemoCue was done by John Peters in the Norfolk Island Hospital. The purpose of the evaluation was to assess the suitability of the instrument for use by the Red Cross Blood Transfusion service in Norfolk Island for the measurement of pre-donor haemoglobin concentration. The instrument correlated very well with the instrument in use in the hospital laboratory. The Red Cross Blood Transfusion service in Sydney was also contacted at this time as they using the HemoCue for a similar purpose. They said that they were very happy with the HemoCue and would recommend its use.

In the early evaluation period the haemoglobins of 11 patients were compared as below:-

NON LIPAEMIC/NON HIGH WBC PATIENTS

A linear regression analysis showed good comparison of the HemoCue with the Coulter STKS. 11 data points were selected giving a Pearsons r (correlation coefficient) of 0.99, $R^2 = 0.99$. The linear regression equation was STKS = 3.08 + 0.99* HemoCue. (Figure 1).

PATIENTS WITH INCREASED WBC

In the early evaluation period only 3 patients with high WBC were tested:

No.	WBCx 10^9/1	HemoCue g/l	Coulter STKS g/l	Spun HB g/l
1	480	76	92*	71
2	364	56	69*	54
3	103	94	101*	94

*These high values were obtained as a result of the turbidity caused by the high white cell counts in these samples.

A Linear Regression was not done on this data as the numbers were too low for statistical analysis.

In the early evaluation period only one lipaemic patient was tested, the result obtained from the HemoCue was very good.

From the limited comparisons above it seems that the instrument compares well in all patient samples that are non turbid. It also seems to compare with the spun haemoglobin method in samples that have a high white cell count.

CONCLUSION

Following our brief evaluation we purchased the instrument and have found it to be excellent for the measurement of haemoglobin in turbid samples, ie both lipaemic and high white cell count samples. We are also using it to measure the haemoglobin of our haemolysates for haemoglobinopathy investigations as it is quicker and more convenient to do them in the HemoCue than to interrupt the workflow on the Coulter STKS.

We are continually monitoring and recording the haemoglobins from the patients with turbid samples and it is our intention to report these findings in a further technical article.

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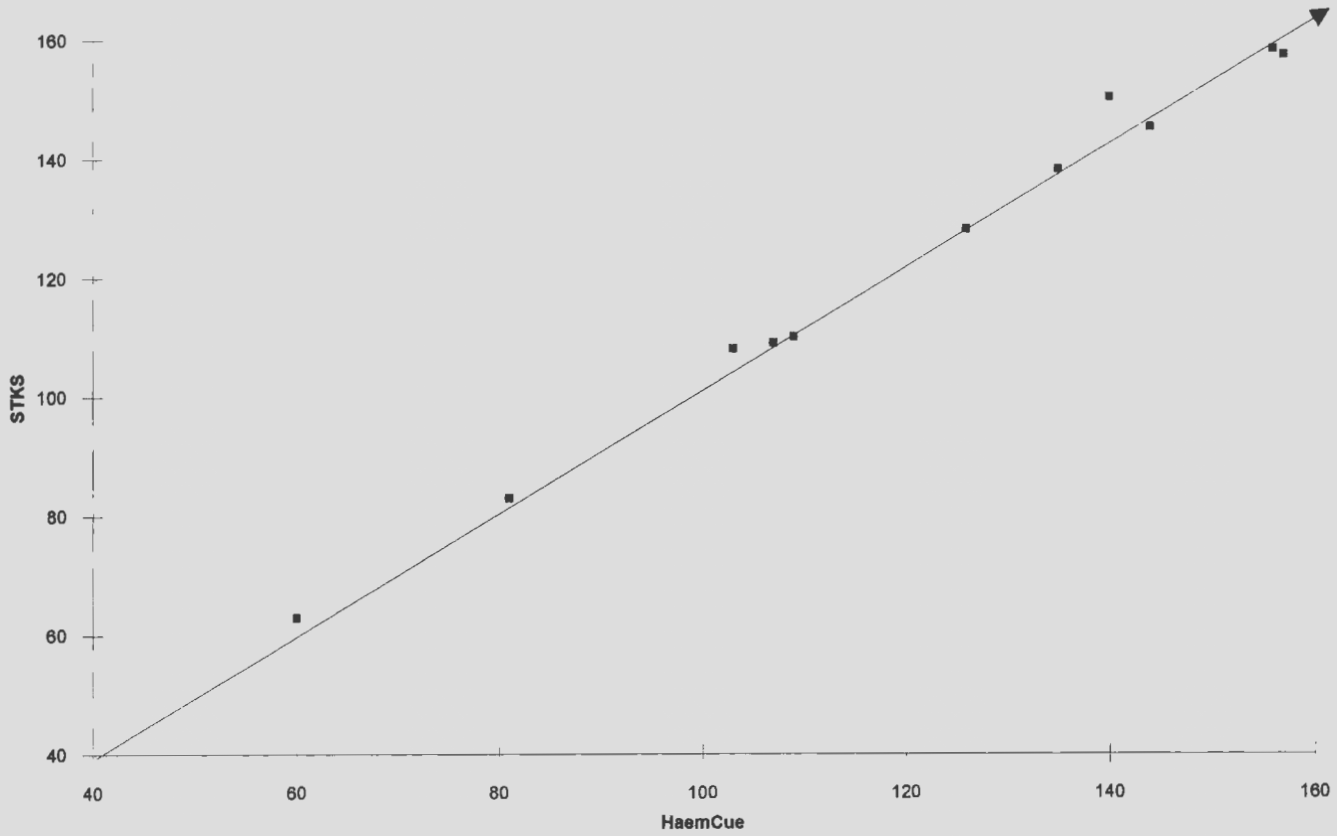

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Continuing Education

In Situ Hybridization

Qurratulain Hasan, PhD
Wellington Medical School, Wellington.

NZ J Med Lab Science 1994; 48 (3) 121-126

In recent years the technique of *in situ hybridization* (ISH) has gained immense prominence and has had a great impact in biology and medicine. This technique has been employed to study plant, animal and human chromosomes, cells, tissues and organs. Genes responsible for common conditions (e.g. Diabetes) and rare diseases (e.g. Huntington's chorea) have been localized to specific chromosomes using ISH. Small changes in chromosome structure which are not detectable by routine cytogenetic procedures are now analysed with the help of ISH. Presence of Papilloma virus, Hepatitis virus and AIDS causing virus have been identified in infected individuals by ISH when other techniques had failed. A number of proteins are known to be involved in the pathophysiology of disease and using specific tissues or cells producing these proteins can be identified. The processes involved in early development (embryogenesis) were an enigma until recently, ISH has helped in understanding these.

Irrespective of which organisms or for what purpose ISH is used it basically involves the detection of specific target nucleic acids (ie DNA or RNA) using appropriate nucleic acids (DNA or RNA) as probes. This paradoxical statement will become clear as soon as we go over some of the basics of molecular biology.

Basic background of molecular biology

The genetic material for most eukaryotes is DNA, which is localized in the nucleus as chromosomes. The information coded in DNA serves as the recipe on which an organism form and function is based. This information has to be passed on to the cytoplasm where the proteins are synthesized – the structural proteins are responsible for giving an organism its characteristic morphology, while functional ones like enzymes and hormones enable it to carry out its metabolic activity. The intermediary molecule which carries the message from the nucleus to the cytoplasma is RNA.

The DNA is a double stranded molecule made up of four nucleotides adenine (A), thymine (T), cytosine (C) and guanine (G). These nucleotides are specifically paired with A pairing with T and C with G using double and triple hydrogen bonds respectively (figure 1). In RNA nucleotide T is replaced with uracil (U).

DNA has the ability to self-replicate using one of its strands as a template to synthesize a new one, making this molecule central to the whole of biology. In spite of DNA having two strands only one of the strands codes for proteins and it is this one which serves as a template for transcription or synthesis of mRNA (figure 1).

The genetic code is a continuous triplet codon where three nucleotides code for a specific amino acid, a string of amino acids form a specific protein.

The passing of genetic information from DNA to RNA to proteins is the central dogma of molecular biology. This is violated in some viruses, whose genetic material consists of RNA. Such viruses possess an enzyme known as reverse transcriptase, which has the capacity to synthesize a complimentary DNA strand using RNA as a template.

The property of nucleotides to pair specifically (ie A=T, G=C) results in complimentary sequences to bind with each other or complimentary sequences to be synthesized. This has been exploited in most of the molecular biology techniques including ISH.

Principles of ISH

Generally, hybridization to detect the presence of nucleic acids can be performed in solid supports, in solutions and on tissue sections or cell preparations. In contrast to other methods ISH allows the identification of DNA and RNA in situ which in latin means its original, natural or appropriate position. Therefore DNA and RNA can be localized in tissue sections, cells or chromosomes.

A critical aspect of the technique involves retaining of target nucleic acids to be detected without being degraded, in single-stranded accessible form for hybridization (figure 2). This is more tricky for RNA hybridization as it gets easily degraded with the commonly prevalent RNases. It is, therefore, essential to ensure that equipment and solutions are RNase free. For DNA hybridization, the nucleic acid has to be denatured to have it in a single stranded form accessible for a complimentary, specific probe to bind it.

Basics of ISH

We have dealt up till now with the target nucleic acids _ DNA and RNA, now let us look at the type of nucleic acids which are suitable probes.

Probes: Probes used can be sequences of DNA or RNA or an oligonucleotide. The oligonucleotides or synthetic nucleotide chains are more popular as they are commercially available and do not require any specific molecular biological techniques to synthesize them. Length of the probe is critical for specificity. If the probes are too short (less than 20 bp) the sequences may find homology with a number of regions resulting in non-specific binding and background staining. Long probes have difficulty penetrating into cells and tissues to find the target material (150 bp may be appropriate).

Probes are labelled with either radioactive or non-radioactive nucleotide incorporation. Commonly used radioisotopes are tritium (^{3}H) and sulphate (^{35}S) while non-radioactive groups employed are digoxigenin, biotin, fluoresceine and rhodamine. Non-radioactive probes are more stable and are increasingly being used as they are safer.

Depending on the type of label on the probes a suitable detection system is employed (figure 2). Autoradiography is employed for detecting radioactive labelling. Non-radioactive labels can be detected by direct or indirect systems.

(a) *Direct systems* have probes covalently linked with signal-generating reporter groups, like fluorescent dyes (fluorescein or rhodamine) and marker enzymes (alkaline phosphatase or horseradish peroxidase coupled to chemiluminescence). The system has a single detection step (figure 3) and is currently being used in gene mapping, chromosome identification and detection of cytogenetic abnormalities.

(b) *Indirect systems* differ from direct-ones by having an additional signal-generating indicator molecule, which couples with the probes modified group. A variety of interaction pairs consisting of modification group and binding partner are available (figure 3).

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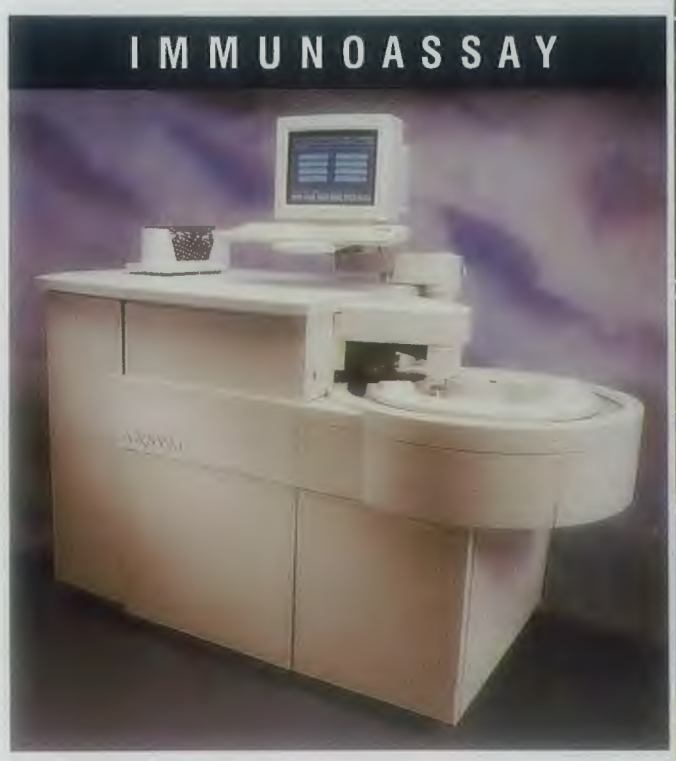

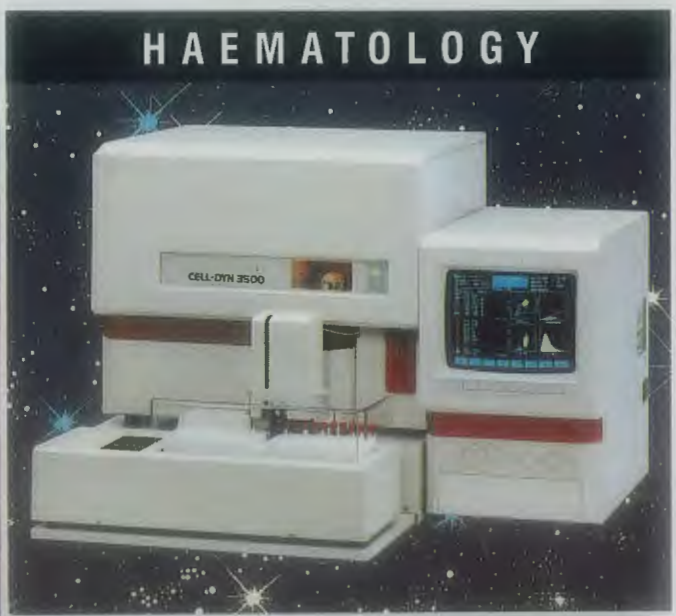

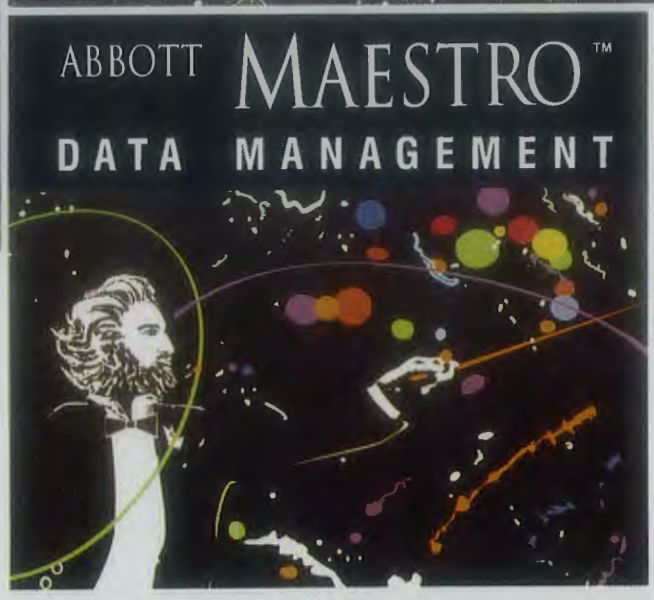

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Common ones are Biotin and Streptavidin, Digoxigenin and a specific antibody. These are extensively used for detecting both DNA and RNA in cells and tissues, as well as to localize viral, bacterial and other parasitic infections.

Pre-treatments: A number of pre-treatments including fixation of material, subbing of slides to prevent loss of the material during the rigorous ISH procedure and protease treatment to allow penetration of probe and its binding with the target nucleic acids are critical for successfully employing this technique. Other pre-treatments like acetylation of sections is used to reduce non-specific binding of the probe to the target material. The appropriate pre-treatment depends on the type of material being used as target and the purpose of the ISH.

Hybridization and washing conditions: The temperature at which hybridization is carried out and the other conditions of hybridization require to be standardized and are based on:

(a) *The type of target and probe:* it is known that RNA - RNA hybrids are more stable than RNA-DNA and DNA-DNA. Therefore higher temperatures and more stringent hybridization conditions can be used for RNA-RNA hybrids.

(b) *Length of the probe:* longer probes are more stable and can withstand stringent conditions, while shorter ones get washed off at similar conditions of hybridization.

(c) *Specificity of the probe:* this should be taken into account prior to determining the hybridization conditions. This means that if a human specific probe is being used on human tissue then more stringent conditions can be used as compared to if a sequence homologous to mouse is used on human tissue and vice versa.

(d) *Sequence of the probe:* this determines the temperature of hybridization because the melting temperature (Tm) of G, C rich probes is higher than that of A T rich probes. This is because G and C are linked by triple hydrogen bonds while A and T by double.

Although numerous labelling and detection kits for ISH are available from a number of companies, the technique is not an easy one. Each laboratory has to find suitable solutions for its specific needs and go through tedious standardization procedures. But, once established the technique has immense potential in the areas of agriculture, basic biology and above all medicine.

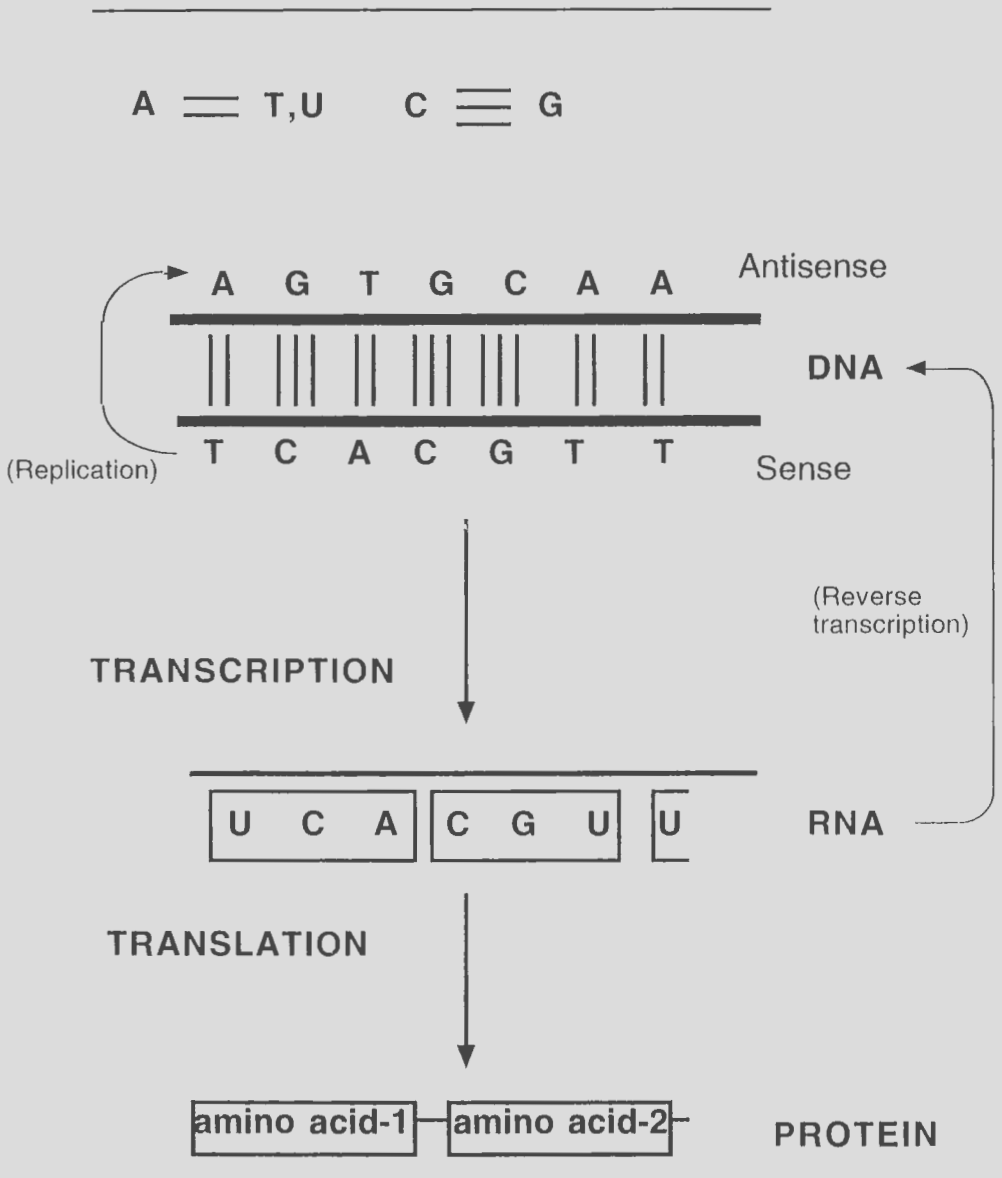

Figure 1

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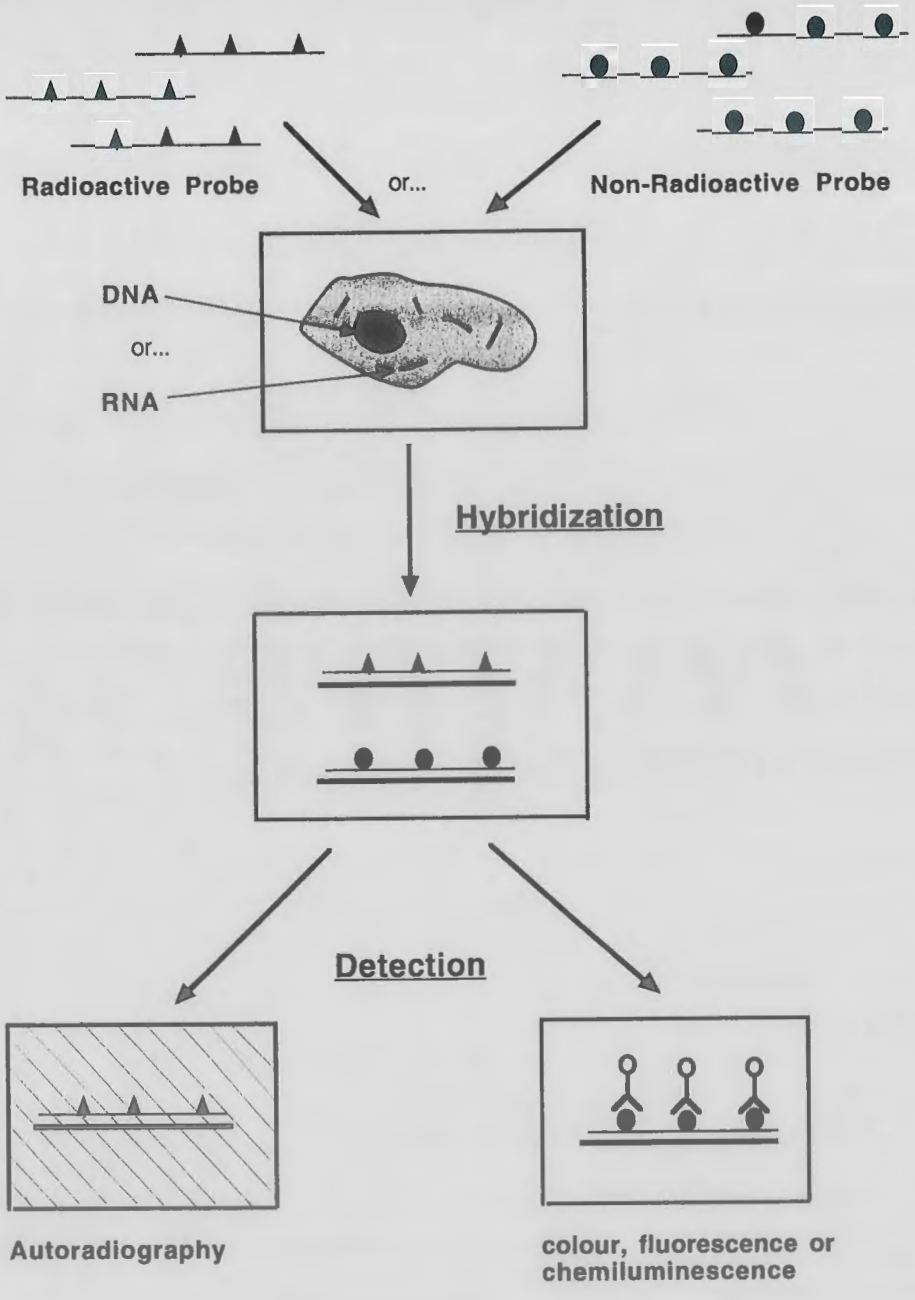

Figure 2

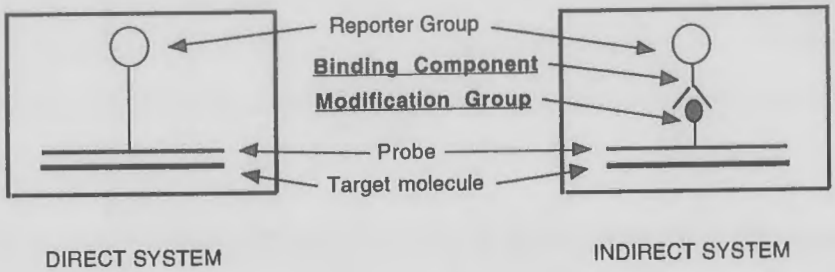

Figure 3.

CURRENT COMMENT

Impact of Technology on the Diagnostic Pathology Laboratory

Dennis Reilly
Diagnostic Laboratory, Auckland

Definition

It should be noted at the outset that there are a number of different definitions of the term "technology" but the definition of Hickson, Pugh and Pheysey (1969) seems most suitable. Technology has 3 parts. *Operations technology, Materials and Knowledge.*

For example, operations technology includes such things as staff to collect specimens and perform the assays, scientific equipment, as well as physical buildings that the laboratory is housed in.

The materials include the specimen containers, chemical reagents and computer report forms.

Knowledge is the third and final aspect of technology. Knowledge is required to collect the appropriate specimen, operate the equipment and deal with exceptional circumstances that inevitably arise.

History

Pathology testing reaches back into antiquity with the scrutiny of urine and its sediments. Then alchemy was applied by Phillippus Aureolus Theophratus Bombastus Von Hohenheim alias Paracelsus (1493-1541) and later more specific procedures were introduced by Robert Boyle (1627-1691). The first chemical laboratory was created in 1842 by Johann Joseph von Scherer in Wurzburg, Germany.

The tests are the means whereby the chemical and cellular pathology of a patient's illness can be elucidated. Analysis can assess the direction and rate of change of a patient's body chemistry in response to the passage of time, the effects of disease or the instigation of treatment.

In the early years of the 19th century laboratory procedures were dominated by morbid anatomy and histopathology. At this time Medical Laboratory Science was an academic subject which centred around tissue samples which were tested by observing macro and microsopically abnormal cells population and morphology.

In 1922, following the discovery of insulin, the impetus was to find a suitable method for estimating blood glucose. This was one of the major factors in the development of the Clinical Biochemistry department.

Initially the laboratories were primitive and poorly staffed. The staff at this time were made up of two groups, the medically qualified pathologist and the scientifically qualified technologist. The technologist undertook a five year apprenticeship training programme, at the bench alongside the pathologist. Considerable expertise and training was needed to perform all the analytical functions to the required high level of accuracy and precision. Technologists would prepare their own reagents, standards and samples before starting an analysis. The assays used simple, cheap and stable reagents.

Despite the limited range of tests, the number of requests slowly increased as clinicians realised how easily they could improve the quality of their patient care, either by using laboratory results to assist in making a diagnosis or as a means of monitoring the response to treatment.

Despite the limited range of tests, the number of requests slowly increased as clinicians realised how easily they could improve the quality of their patient care, either by using laboratory results to assist in making a diagnosis or a means of monitoring the response to treatment.

The requesting clinicians because of the limited menu of tests were conversant with the interpretation of results themselves and able to compare and evaluate them with the clinical signs of their patients.

The scene stayed much the same until the 1960's when it underwent radical and profound change.

Mechanisation

Mechanisation was introduced in the 1960's after the invention of the Autoanalyser in 1957. Whereas in the past, limitiations of staff availability and inclination decided where when and what tests would be performed, the Autoanalyser with its processing rate of 40 test per hour opened up the prospect of test volume being limited only by the number of machines. Increasingly control and discretion were removed from the staff and placed directly onto the automated equipment. Many felt that this control had robbed them of their freedom and enthusiasm for work. Some preferred the more manual departments because of this.

Prior to 1950 the output from the lab was limited by the few numbers of skilled technologists. By using automation the numbers of tests mushroomed and lead to an exponential growth without a corresponding increase in labour.

Firstly Clinical Biochemistry was involved, then Histology Haematology and more recently Microbiology.

With the advent of automation the number of tests per technologist increased and the skills of the technologist needed to change to absorb the new technology. Almost simultaneously with the advent of technology was the introduction of a new work group the Laboratory Assistant. Increasingly analytical functions are done by one or two machines with relatively unskilled staff feeding the samples in. These assistants are semi-technically trained and do a lot of the setting up tasks, like centrifuging and loading samples onto carousels and placing onto the Autoanalysers. Appendix shows the trend of employment of laboratory assistants over the past 30 years to now include 40% of the workforce.

Technologists took to the autoanalysers with enthusiasm and used their expertise to convert manual methods onto the autoanalysers. Many technologists over the years have transferred from the traditional laboratories into the commercial companies to create new autoanalysers or to write new software.

Many technologists now work for these scientific companies and modify the autoanalysers to a customer's specific needs before being installed into the laboratory. For example a robotics sampler may be set up for infectious disease testing in one laboratory and as a blood grouping analyser in the next.

Another area that became automated was the laboratory office. Many laboratories were overwhelmed with the increasing workload

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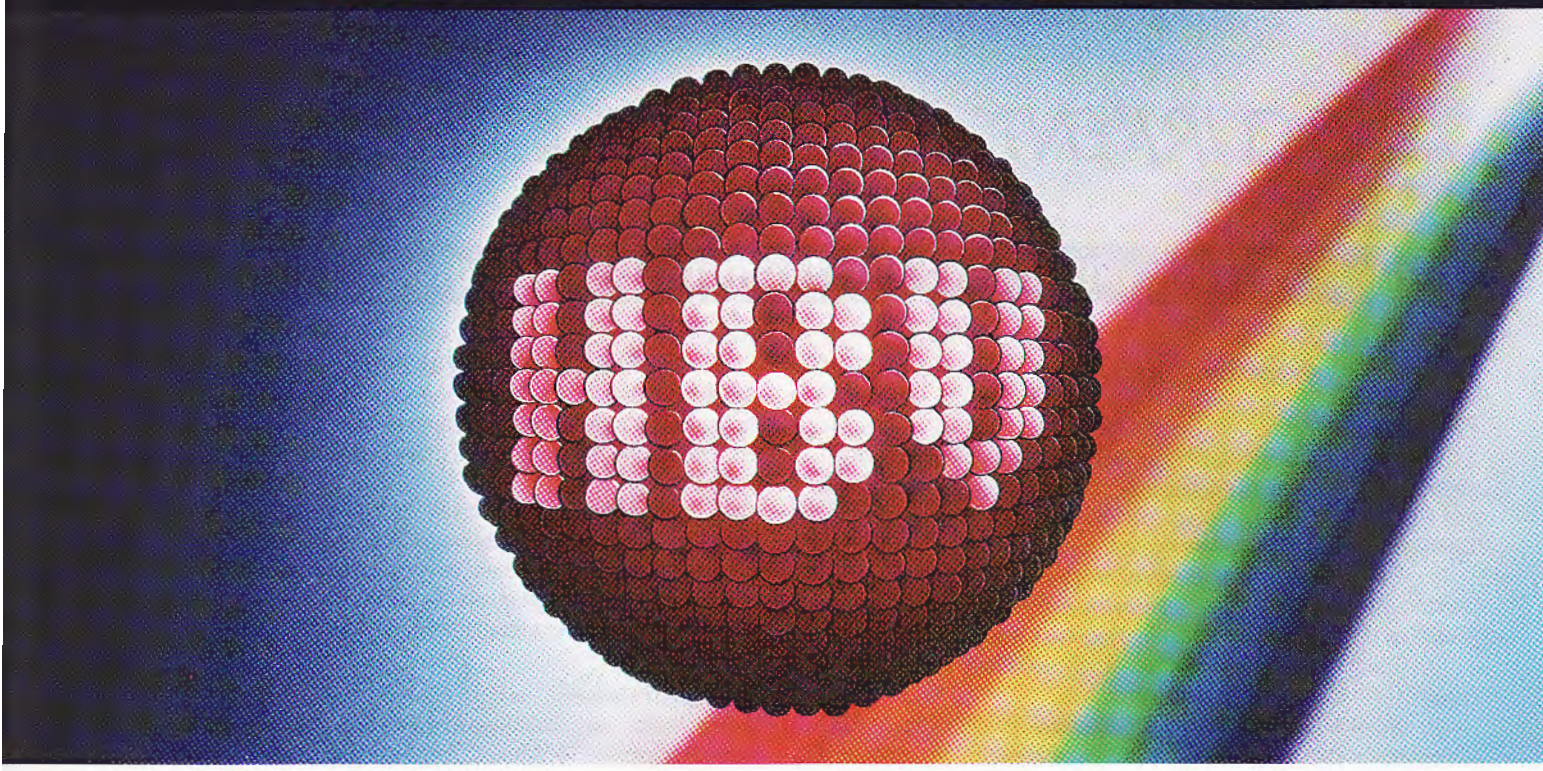

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and used the new computer technology to write their own software to improve the clerical reporting of the increasing workload. Computer terminals initially were only in the laboratory but are now in wards, operating theatres and Doctor's surgeries. Because of the critical shortage of programmers who could bridge the gap between the complexities of the laboratory and the software many technologists upskilled their knowledge and moved into the specialised area of computer programming.

More recently there has been the development of an area of computer technology known as "expert systems". The view is held that all or most humans mental tasks can be performed by sophisticated software packages. It is said to be superior because humans cannot cope with the sheer volume of knowledge that needs to be sifted to arrive at a decision.

The Garvan Institute of Medical Research in Sydney has written an expert system that can interpret endocrine diagnostic tests and write medical care reports.

An argument here may be that personnel have been de-skilled by this move from judgement by laboratory professionals to that which makes nurse diagnosis or even patient self-diagnosis to automatic diagnosis by the auto analyser/computer combination possible.

Materials

Later in the 1960's came the invention of the methodology of Radio immunoassay (RIA). Once again the impetus was the diabetic patient.

Yallow and Berson, in developing RIA for *quantitation* of Insulin were awarded the Nobel Prize which rightly reflects the huge advance this was to Medical Laboratory Science. The reagents used were highly complex, with simple chemicals being replaced by expensive protein antibody solutions, antigen substrates and radioisotopes.

Reagent preparation moved out of the laboratory and into the scientific companies with the introduction of pre-packaged kits. The kits gained widespread acceptance because of the minimal demand it made on technical skills and understanding for satisfactory performance. The Companies made reagent kits that operated exclusively on their type of automation. All the technologists had to do was simply place specimens and reagents onto the instrument.

Organisation

Laboratories have traditionally had a formal organisation which is relatively large scale and highly structured, sometimes described as bueaucratic.

There was a strict division of labour by expertise, hierarchical in that each person was accountable to a superior, and a consistent system of rules to ensure uniformity and impartiality. Employment and promotional prospects within the system were based on qualification. This centralisation of control, had a number of negative consequences for staff as well as the laboratory itself. Serious dissatisfaction was noted in the mid 1980's when the laboratory was extremely bureaucratic and staff resignations were at an all time high.

The current departmental divisions, which have evolved over the past seventy years arose because of the specialist expertise needed to perform the analytical functions. These specialist departments are now under threat. The new computer controlled analysers are capable of performing a wide range of tests which formally were separated into several different departments and have integrated what previously was fragmented and differentiated.

The new technology is shaping and enabling this organisational change. Departments are now amalgamating, for example Biochemistry/Immunology, Virology/Immunology. The new technology with its all-encompassing test menu will bring the departments together.

Conditions that have been found to promote adaptability, innovation and change include the decentralisation of power, low levels of formalisation, equity of rewards and high emphasis on job satisfaction. The skills and knowledge of staff members are amplified by having them rotate through a variety of departments during their careers, thus building their skills and knowledge. This knowledge is invaluable for co-ordinating activities among different departments of the laboratory and is a more efficient mechanism for integrating service than any bureaucratic structure. Greater reliance on staff knowledge and decision making ability also reduces the need for front line supervisors and middle level managers.

Knowledge

The skill acquired by the technologist during their initial years of training are no longer enough to see them through their working lives.

The arrival of new technologies, new methodologies, and the huge increase in workload have forced the technologists into the process of continuing education.

Initial training has now moved into the Universities with a 4 year baccalaureate course. The BMLSc graduate will have a higher level of medical knowledge as well as scientific skills to practice in a high tech laboratory in the 1990's. However the biggest need for the future will be for continuing education at seminars, workshops and in the workplace. This type of knowledge cannot be gained before entering the laboratory, so organisations such as the NZ Institute of Medical Laboratory Science will be required to provide this service on an on-going basis.

We see some laboratories staging lectures for General Practitioners and their Nurses, for the purpose of providing up to date information on a variety of new tests, and the MLTB is about to introduce a Continuing Competency Programme to ensure that technologists have a more current knowledge level before issuing their annual licence certificate.

Conclusion

Technology has transformed the skill requirements of workers in the pathology Laboratory Profession. Automation will continue to perform a large number of unrelated different tests with very little intervention. Continuing research will make laboratory instruments easier to operate and more efficient. Old tests will become easier. Each newer and more complex tests will be added. In the future it is unlikely that every instrument will be run by highly trained technologists. Instead, laboratories will be staffed by assistants under the direction of a limited number of registered technologists. These supervising technologists will have the expertise to solve instrument problems and to address general issues of medical and laboratory understanding. The ability of new automation to perform more tests with less supervision, as well as a general increase in cost pressures, will continue to reduce staff input per test and require continuing improvements in productivity.

In contrast to the past, nowadays the modern laboratory with its vast menu of tests, sees the clinician at a disadvantage to understand fully the significance of the full range. The technologist will need to be able to discuss these tests and assist the Clinician in choosing the appropriate tests for the benefit of the patient as well as holding costs within the health system.

The technologists of today, using their sound education base, will graft on other appropriate qualifications to ensure they remain an information centre for the laboratory medicine team. Technology has changed the characteristics of the modern technologist. The role is more demanding than in the past and more varied, interesting and rewarding. Methodology advances, such as biosensors have seen some tests moving from the laboratory and into the Doctors surgery and Hospital Wards and performed by Doctors, Nurses and indeed Patients.

The technologist of the 21st century will be expected will be expected to have an extensive knowledge of laboratory medicine, and more importantly be innovative and adaptive to changes.

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OBITUARY.

GORDON WALLACE McKINLEY, F.N.I.M.L.S.

Born Helensville, North Auckland, October 4 1912
Died Waipukurau, May 15 1994
Retired Bacteriologist, Waipukurau Hospital.

Mac's passing severs an important historical link for the Institute. He served on council from its inception in 1945/46 until 1956/57 as council member, secretary, Vice President and then from 1955 to 1957 as the Institute's fourth president. He was created a Life Member in 1958. He was a strong advocate and supporter of the Institute and the profession through his long working life. He was for many years the senior examiner and moderator for the Health Department examinations.

He attended Ruawai school in the North where his father was headmaster; later Hikurangi Maori Boy's College in the Wairarapa where he was the only Pakeha pupil. His final years of education were at Hasting's Boys High School where he was Head Prefect from 1930-32; captain of the first fifteen and captain of the first eleven. His contribution to this school was recognised when he was guest of honour at the 1993 prize giving.

He began his laboratory training at Napier Hospital in 1933 and completed bacteriology training at Auckland Hospital. In the final examination in Dunedin in 1939 he gained the highest mark in New Zealand. He was appointed Charge Bacteriologist to the Waipukurau Hospital in 1943. This was an important post as at that time the Pukeora Sanitorium near Waipukurau was regarded as the major TB hospital in New Zealand with over two hundred patients. Gordon was recognised as a leading authority on Tuberculosis microbiology in this country. Despite handling thousands of positive TB specimens over the next 15 years his mantoux never converted. This was a tribute to his obsessive attention to detail and sterile technique and the use of his clean hand/dirty hand approach.

This period also saw the introduction of chest surgery at Waipukurau hospital with visiting sugeons from Wellington. These were major operations being performed with limited resources in a small country hospital. Jenny Shipley would not be pleased. The blood transfusion demands at this surgery in a small rural community meant that Gordon had to set up and organise a panel of donors, bleed the donors then cross match the blood.

When I joined the staff in 1961 it was still commonplace to call donors during the middle of the night, collect the blood; then go to the lab next door for the two hour cross match. His son Duncan recalled in the funeral oration, giving blood on many occasions in the middle of the night when 'Dad' could not contact a suitable donor.

His canny Scot's heritage meant that the lab was run on a "shoestring" budget. All microscope slides were reused and pasteur pipettes were made in the laboratory. Equipment in the 1960's was maintained in operating condition long after it had been replaced in other New Zealand Hospital laboratories. The laboratory at Waipukurau Hospital was a working museum. Consumable supplies rarely exceeded £500. He was especially fond of all things Scots. For many who knew him his trademark was the ever present pipe. Any visit from him resulted in ashtrays overflowing with dead matches and any walk along the street saw Gordon wreathed in smoke looking for all the world like a Ka locomotive on the main trunk line. He was a real train enthusiast. In the lab, glass petri dishes were used as ashtrays and the pipe was tapped out on the outside window sill. Carpenters at the hospital replaced this windowsill twice during his time in the lab.

He knew and was respected by the leading pathologists of his day – D'Ath, Gilmour, Pearson, Lynch and Pullar; however his dealings with Laurie Cameron of the Health Department were especially significant as they set salaries for Bacteriologists over a few drinks in the Wellington club after a day of negotiation at the Department. Not surprisingly, on his retirement Gordon was among a handful of Grade five technologists in the country.

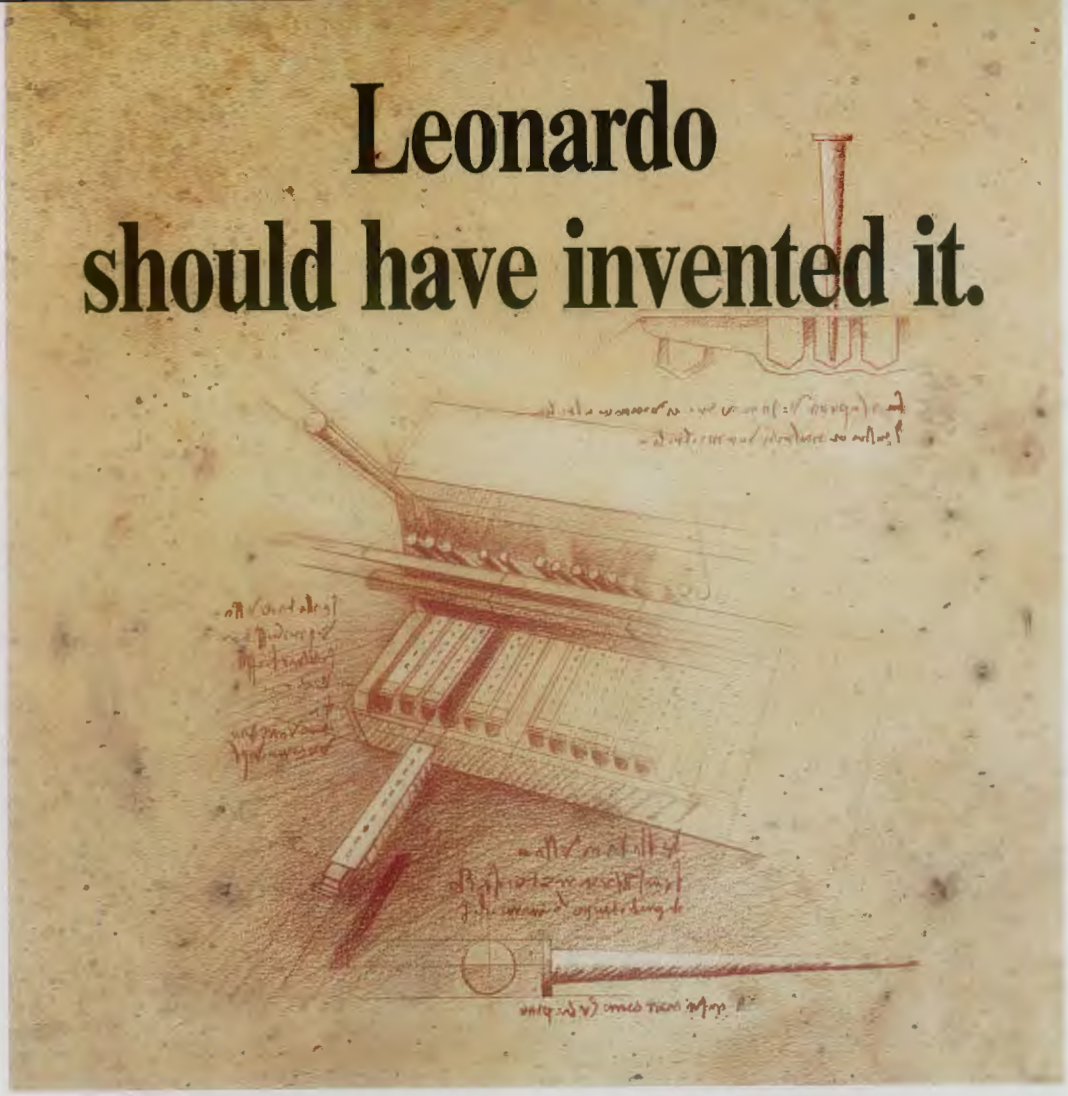
The early years of the Institute saw council members expected to pay their own way for attending meetings. There would be a reluctance for anyone to serve on council if that were the case today. Gordon used to catch the train to Wellington from Waipukurau and on many occasions had to ring the station manager to get the train held for a few minutes as he was still in the lab when the train was due to leave.

Gordon McKinley gave me and many others a strong grounding in Medical Laboratory Science and a deep commitment to the Institute for which we should all be grateful.

The profession and the Institute are poorer for his passing. Our symathy is extended to his wife, Peg, sons Fergus and Duncan and their families.

Colvin Campbell

Leonardo should have invented it.



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Editorial

Publishing in the Journal

Rob Siebers – Editor
Wellington School of Medicine

Our Journal is a peer-reviewed publication. When potential authors submit their manuscript to the Editor it is initially scanned to see that it more or less complies with the Instructions to Authors. It is then determined which major discipline of Medical Laboratory Science the paper addresses and the manuscript is then sent to at least two referees with expertise in he relevant discipline for constructive criticism and comments. Upon receiving the referees' reports the Editor then decides whether the paper is suitable for possible publication in the Journal.

It is most unlikely that a submitted manuscript will be published in the Journal without some changes being necessary. The referees may require clarification of certain points or require more detail of methodology. Alternatively the Statistical Adviser to the Journal may point out that the statistical analysis is either inappropriate, wrongly interpreted or lacking. Additionally the Editor may require rewriting of various sections to make the paper clearer to readers, require the author to include subheadings, rewrite the reference section in the required "Vancouver" style, or redo figures and tables for clarity.

This process should not be seen as a dark plot by the Editor to prevent authors from publishing their paper in the Journal. It is intended to ensure that the paper is of quality and interest, and is readable by the members. The comments of the Editor, referees and statistical adviser should be seen as being of help to you as author to present your paper in the best possible light.

If you as author require help or advice regarding your paper prior to submittance to the Journal feel free to write or phone me, or any member of the Editorial Board whose names are published in this and subsequent issues of the Journal. They will be more than willing to give you expert advice and help. Although brief and concise instructions to authors will appear in each issue of the Journal as Editor I would like to emphasise are what I consider to be three key issues. They are:

1. The Abstract. The Editor, referees and readers of the Journal invariably read the abstract first to determine the objectives, methods, results and conclusions of the study. If written in a clear and concise style the abstract makes the paper that follows of potential interest to readers. The abstract should be able to stand on it's own apart from the text of the paper.

2. Tables and Figures. As with the abstract each table and figure should be able to stand on it's own. They should be brief, precise and uncluttered with unnecessary wording or symbols. The text for both tables and figures should be brief and not just word repetition as it appears in the results section. The accompanying text for the tables and figures should be typed on a separate sheet.

3. Methods. This section should be as brief as possible yet comprehensive enough for any experienced reader to duplicate the methods described. Not only should analytical methods be described here, but also selection of subjects, response rates, names of suppliers of chemicals, drugs and equipment (in parenthesis), and where appropriate the type of statistical analysis and significance levels.

It is hoped that the above mentioned is of use to potential authors. It certainly makes the life of the Editor easier if these basic points and instructions to authors are followed as closely as possible. There is no greater satisfaction for authors than to finally see their paper in print after many hours (weeks, months) of conducting the study and writing it up for the Journal.

Our Institute manages many awards for authors who ultimately publish in the Journal (provided they are financial members or associate members of the NZIMLS). Every two years there are the Roche Diagnostic Awards for the best Clinical Chemistry and Microbiology papers, the Pacific Diagnostics Award for the best Haematology or Transfusion Science paper, the NZIMLS Award for the best paper in subjects not mentioned above, the NZIMLS Student Paper Award, and the Hilder Memorial Award for the best Technical or Short Communication. Over and above these awards is the Med-Bio Award for the best paper in each issue of the Journal.

I hope the membership will make my role as Editor both easier and more difficult! Easier by complying with the instructions for authors, and more difficult by deluging me with your manuscripts.

Letter to the Editor

Dear Sir

As a former editor of the publication that has now become the *New Zealand Journal of Medical Laboratory Science*, I read your editorial in the May 1994 issue with some interest, and no small measure of compassion.

It is clear you are fully conscious of the challenge facing you as the newly appointed editor, and of the manifold difficulties that have hampered the efforts of your predecessors. It isn't going to be easy, for sure; and you'll no doubt find your new obligations to be a drain on your leisure time. Never doubt, however, the importance of your task, and the worthwhile role played by the *Journal* in facilitating communication between medical technologists in New Zealand. I used at times, I confess, to curse the lack of support I felt I was getting. I even wondered, on occasion, whether I was not merely wasting my time in striving so hard to keep our modest publication going. Yet I felt overall that it would be letting down a proud tradition if I simply threw my hands in the air and gave up – a tradition that began with the late Douglas Whillans not only editing the thing, but *printing it himself*!

Each of us that has borne the editorial duties you have now assumed has contributed something in terms of new ideas, and I am sure you will be no exception. There will no doubt be those that pay only lip-service to the principle of supporting your endeavours, but among those that express a desire to be helpful there will undoubtedly be some that really do come through. There was for me, I know, as I'm sure there was for each of my successors. Had it not been so, the *Journal* might have foundered years ago.

Keep up the good work! With best wishes,

John Case *FIMLS*
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Editor
Rob Siebers
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Membership Fees and Enquiries
Membership fees for the year beginning April 1, 1994 are:

For Fellows – $88.40 GST inclusive

For Members – $88.40 GST inclusive

For Associates – $33.80 GST inclusive

For Non-practising members – $33.00 GST inclusive

All membership fees, change of address or particulars, applications for membership or changes in status should be sent to the Executive Officer at the address given above.
Members wishing to receive their publications by airmail should contact the Editor to make the necessary arrangement.

NEW ZEALAND INSTITUTE OF MEDICAL LABORATORY SCIENCE 1994 CALENDAR

17 August	Annual Report and Balance Sheet to be with the membership (14 days prior to AGM)
24 August	Ballot papers and proxies to be with Executive Officer (7 days prior to AGM)
29/30 August	Council Meeting – Hamilton
31 August	AGM and SGM – Hamilton
31 Aug-2 Sept	Annual Scientific Meeting – Hamilton
2 November	QTA examinations
9/10 November	Specialist Certificate examinations
17/18 November	Council Meeting

Membership Sub-Committee Report May1994

Since the February meeting there have been the following changes:

	11.05.94	14.02.94	05.11.93	19.08.93
Membership	1172	1177	1178	1235
less resignations	35	7	2	16
less G.N.A.	21	4	4	10
less deletions	-	-	-	118
less deceased	-	-	1	-
less duplications	-	-	-	1
	1116	1163	1171	1108
plus applications	21	7	4	66
plus reinstatements	1	2	2	4
	1138	1172	1177	1278
Composition				
Life Members (Fellow)	12	12	12	10
Life Member (Member)	8	8	8	8
Fellow	20	20	20	20
Member	662	683	684	686
Associate	355	367	371	372
Non-practising	55	56	56	56
Honorary	26	26	26	26
Total	1138	1172	1177	1178

Applications for Membership
S. JONES, Medlab Auckland, R. MASON, Medlab Taranaki, G. DENNIS, Christchurch.

NZIMLS

Council
Elected by members.

Membership
Voluntary – open to all laboratory workers.

Purpose
To promote professional excellence in medical laboratory science.

Main Functions
1. Represent and act where appropriate in the interests of the profession and its members.
2. Support ongoing education:
   - SIG workshops/seminars.
   - Annual Scientific Meeting.
   - South Pacific Congress.
3. Publish a scientific journal newsletter
4. Conducting examinations:
   - Fellowship, Specialist and QTA levels.
5. Develop and maintain contacts with kindred societies overseas:
   - Membership of the IAMLT.
   - Support of the PPTC.

Responsible to
The members of the Institute

MLTB

Board
Appointed by Minister of Health.

Membership
Compulsory for all those who practice medical laboratory science in New Zealand as medical laboratory Scientists.

Purpose
Act as guardians of the public interest in professional standards in medical laboratory science.

Main Functions
1. Maintain register of recognised technologists.
2. Issue the Annual Practising Certificate.
3. Establish and maintain the recognised competencies required for registration.
4. Conduct examination of certificate level.
5. Consider concessions to registration.
6. Maintain disciplinary functions set out in legislation.
7. At all times, act in the interests of the public and patients.

Responsible to
The Minister of Health

THE NEW ZEALAND INSTITUTE OF MEDICAL LABORATORY SCIENCE (INC.)

Title	**Jim Le Grice Award**
Nature	An annual award in memory of Jim Le Grice to sponsor a full time student, qualified staff technologist or qualified technical assistant to the Annual Scientific Meeting.
Eligibility	1. Any student who is a member of the NZIMLS and in full time tertiary education. 2. Any qualified technical assistant or staff technologist with less than 5 years total work experience. (Work experience to be verified on application form).
Conditions	No conditions apply to student applications. However, qualified staff will present a paper or poster at the Annual Scientific Meeting.
Applications	Applications should be completed on the official application form published in the NZIMLS Journal and available from the Executive Officer, NZIMLS, PO Box 3270, Christchurch.
Selection	Will be made by ballot by the convenor of the NZIMLS Awards Committee.
Amount	The prize awarded will vary yearly and will consist of travel to and from conference, accommodation and registration with the successful applicant making all arrangements.
Term of Award	Initially offered in 1995 and subsequent 9 years with a review at that time.

PACIFIC PARAMEDICAL TRAINING CENTRE (P.P.T.C. – News in Brief)

The activities of the P.P.T.C. for this year 1994 include the following:

February/April

A course on Blood Bank Techniques and Blood Bank held in Wellington at the P.P.T.C.

April

The Tutor Co-ordinator Mike Lynch at the invitation of the W.H.O. visited Western and American Samoa with Dr Rolland Farrugia, a W.H.O. Consultant for Tuberculosis and Leprosy. The purpose of Mike's inclusion in the visit was to work in collaboration with the Governments of Western and American Samoa (W.A.S.) to:

1. Assess the Laboratory work for Leprosy and Tuberculosis.
2. To train Laboratory Technicians in standard techniques for slit-skin smears and Tuberculosis.

The project was funded by the W.H.O. and the Pacific Leprosy Foundation. Both organisations have a commitment to the elimination of Leprosy in the South Pacific. The worsening of the Tuberculosis epidemic in many parts of the world has lead the W.H.O. to re-assess the current Tuberculosis epidemic in many parts of the world has lead the W.H.O. to re-assess the current Tuberculosis control strategy and to develop a new frame-work for Tuberculosis control. Consultants in the South Pacific area involved in the Leprosy elimination programme are being asked to also assess the Tuberculosis situation.

- A video tape on the 'Preparation of Blood Components, Serum and Cells' is in preparation and currently is up to the editing stage. This video will be used for teaching purposes both at the P.P.T.C. and in the Blood Banks and Laboratories of the Pacific Islands.
- The second Western Samoa Training Programme for Laboratory Technicians is now in year two and is progressing well.
- Four students from Pacific Island Laboratories are currently in New Zealand on attachment training. They have been placed in Laboratories in the Wellington area.
- A Laboratory Technicians 'Update Course' is to be held in September/October at the P.P.T.C.
- The P.P.T.C. will provide a consultant to the Laboratory in Rarotonga for two weeks in July/August.
- Negotiations re providing 'On-site' training in Bhutan, Papua New Guinea and Malaysia are in progress of development.

THE GLOBAL TUBERCULOSIS EMERGENCY

In 1993, W.H.O. declared the resurgence of Tuberculosis (T.B.) a global emergency. Tuberculosis is the leading cause of death due to a single infectious agent. No country can afford to ignore the threat of the current Tuberculosis epidemic to the health of its population and to its economy and development efforts. Nearly 90,000,000 new cases of Tuberculosis will emerge during the next decade, the majority amongst the age group 20-49, which represents men and women in their most productive age. Out of them, up to 30,000,000 will die unless the attitude to the global Tuberculosis problem is changed radically. It has to be realised that the high mortality due to Tuberculosis is often the result of inadequate control measures and neglect of the disease.

The revised strategy is simple ... provide efficient treatment to – at least – all sputum smear positive Tuberculosis patients. However, the implementation of this strategy requires the full commitment of Governments and Health Staff at all levels.

To ensure progress towards the adoption of this strategy, the W.H.O. Tuberculosis programme recommends a frame-work to all Member States where Tuberculosis is a major public health threat. The adoption of this frame-work is particularly urgent in countries confronted with the H.I.V. epidemic, which will result in an increasing number of Tuberculosis cases and specific operational problems. It is critical that the frame-work be fully understood and widely implemented to deal with the present Tuberculosis emergency and to prevent a potentially uncontrollable epidemic of multi-drug resistant Tuberculosis. The frame-work identifies key operations and provides essential indicators for monitoring T.B. programmes.

IRISH-SAMOA CONNECTION

An organism currently having a devastating effect on the Western Samoa Taro crop (Phytophthora colocasiae) has a long and terrifying pedigree. A close cousin of this disease is the infamous Potato Blight which resulted in a famine that killed 30% of Irelands population in 1840 and forced many to emigrate.

It is expected that 40% of Samoa's crop of Taro this year will be wiped out. The Western Samoan Department of Agriculture reports the disease has spread to every district in the Island of Upolu and is reported on 60% of the Island of Savaii. The spread of the disease is remarkably rapid. The production of Taro will need to be stopped for one year to enable complete field sanitation to be undertaken. This will have a devastating effect on the Western Samoan economy.

EXCITING AND PRACTICAL RESEARCH

An Agricultural Economist Dr Ian Etherington, with the Research School of Pacific Studies (R.S.P.S.) and Australian National University has found coconut oil may be a viable replacement for diesel oil. His latest research indicates villages may be able to extract the valuable oil themselves. The discovery that locally produced coconut oil can fuel power generators, trucks and outboard motors is exciting news for all the Pacific, and especially for small communities struggling to generate cash income.

Dr Etherington says 15-20 coconuts will make 1 litre of coconut oil. 'That's enough to drive a vehicle for 10 kilometres, at a ratio of 1 kilometre to 2 coconuts. Or it could run a motor to give 200 hours of fluorescent light, or power a 100 litre fridge for 10 days, or it could power a small 5 hp outboard motor for 40 minutes'.

Local production of coconut oil would mean that Pacific Island communities can be more self-reliant, and that they can cut fuel bills and reduce environmental pollution.

CAUSE FOR CONCERN – KAVA DRINKING

Excessive Kava drinking is being linked to health and nutritional problems, a drop in production of food crops and pressures on family life. Kava has long been central to life in the South Pacific but not all Islanders are happy about the wide-spread use of the regional 'grog'. Many church leaders, Politicians, Health Workers and Government Officials in Fiji are becoming increasingly concerned about the heavy consumption of Kava, which was once restricted to men of senior social rank.

Kava is a relaxant and soporific, inducing a sense of peaceful well-being. It does however, have addictive qualities and when drunk in excess, causes lethargy and a loss of appetites.

Excessive Kava drinking is believed to adversely affect a man's health and appearance – people become lazy with the consequent neglect of gardening and other responsibilities. Some nutritionists believe Kava use has contributed to a general decline in public health in Fiji through a drop in the production and consumption of traditional root crops. Because it is lucrative, farmers often plant Kava instead of food crops.

AMERICAN CRUISERS PROVIDE MEDICAL AND DENTAL ASSISTANCE TO COOK ISLANDS

Tooth brushes, tooth paste and transportation were donated by several American yachties transiting the Cook Islands. The donations will go to school children on the Islands of Penrhyn, Pukapuka and Manihiki where tooth brushes and paste are not available for purchase in the Islands stores. Dr Wolfgang Losacker initiated a W.H.O. sponsored medical survey of the outer Islands checking for Tuberculosis, Leprosy, Heart Diseases, Skin Disease and Dental problems. He was given the opportunity to join an American Ketch owner aboard the "Mahina Tiare' and select Islands in the Northern Cooks they should visit. Dr Losacker was able to meet all the residents of Penrhyn and Pukapuka and other patients on Manihiki.

The programme was such a success thanks to John Neil, owner of the "Mahina Tiare' that Dr Losacker will team up again with him this year. This visit will mark the fifth time in ten years that "Mahina Tiare' has brought needed and requested medical supplies to the isolated Northern Cook Islands.

In addition to tooth brushes and tooth paste, the isolate Northern Cooks are in need of Oral Antibiotics such as Penicillin, Ampicillin, Tetracycline, Bactrim, Cloxacillin, Augmentin, Cephalosporin, Gentamycin, Prednisone, Hydrocortisone, Panadol and Sterile dressings, gauze and other items.

Contributions can be shipped to Dr Wolfgang Losacker via Air Mail and care of Rarotonga Hospital, or to 'Mahina Tiare' Sailing Expeditions, Box 1596 Friday Harbour, WA98250 206-378-6131. Used school text books are another item of great value to the Cook Islanders.

NEW ZEALAND MEDICAL TECHNOLOGIST WITH CHINESE JUNK RIG SITED IN VANUATU

Michael Churchhouse formally Charge Technologists Cytology Unit National Women's Hospital was reported recently to be in Vanuatu and heading north of the Equator. Michael and wife Judy left Auckland in June last year. They have been cruising the Pacific aboard 'Shantung', a 31 foot Lidgard Design, with a Chinese Junk Rig. Michael reports he has had great fun in the Vanuatu group of Islands, transporting live chickens, bags of produce and more than 50 men, women and children, nursing babies and guitar players across Havannah Harbour.

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NZIMLS CONTINUING EDUCATION

SPECIAL INTEREST GROUPS

Liftout

Microbiology

Special Interest Group

Convenor: Jan Deroles – Main
Contact Address: Medical Diagnostics
Palmerston North

MSIG TELECONFERENCE

Thursday 16th June, 2:15 p.m.

The participants included the full committee as follows:
Jan Deroles-Main (Convenor)
Janet Wilson (Treasurer)
Mary Carr (Secretary)
Shirley Gainsford
Dave Reilly
Dave Scarrow
Several items were discussed as follows:

1995 Seminar: It was decided that we should hold a one day seminar in March 1995. The venue is again to be in the central North Island, but the date is to be arranged so as to avoid a clash with the South Island seminar.

The possibility of holding a second seminar in association with the Microbiology Society conference is to be further considered at our meeting in Hamilton (see below)

MSIG Committee Meeting to be held at Conference – arranged for Thursday lunchtime – all welcome.

Publicity: Agreed that a further Newsletter should be sent out when dates for coming events are decided. A poster will be displayed at Conference, giving information about the activities of the MSIG.

Microbiology Society Conference: This is being held in Dunedin next year. The program will place more emphasis on clinical microbiology than has been the case previously. The question of putting some advertising in our journal and/or newsletter will be discussed further.

Guidelines for T.B. Diagnostic Testing in New Zealand: It was agree that the MSIG should look into the formulation of some guidelines for laboratories undertaking this work.

Taupo Seminar Prize: Heather Laird, who was awarded the prize for best presentation (which was to include presenting at Waikato), is unable to take up as she is understandably busy preparing for her specialist level exam. An alternative arrangement was discussed.

South Island Representation: It was agreed that a second representative from the South Island would be a good idea.

M.S.I.G. Journal Club

Would you like to see the contents pages of current Microbiology journals. For $20.00 you will be sent a copy of the contents pages for each of twelve different journals for all issues for 1994.

To Join:
Send Name, Laboratory, Address to Philippa Skellern, Microbiology Department, Medlab, P.O. Box, 4120, Auckland
For Members of N.Z.I.M.L.S. only
Copies of the contents pages of twelve different Microbiology journals can be sent to you for all copies for 1994.

BOOK REVIEWS

"WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction" Third edition. World Health Organisation. Published by Cambridge University Press 1992. IBSN 0 521 42168 3 paperback.

This book is a revised version of the original laboratory manual published in 1980. Its aim is to encourage the use of standard procedures in the analysis of human semen. The book is divided into 5 sections. Section 2 which deals with the collection and examination of human semen is the section of most interest to general clinical laboratories. It covers sample collection and delivery, the macroscopic and microscopic examination, tests for spermatozoa antibodies and includes optional tests such as culture and biochemical analysis. Research tests such as computer aided sperm analysis are briefly mentioned.

The microscopic examination includes both wet film using phase contrast microscopy and stains (Giemsa, Papanicolaou, Shorr and Bryan Leishman). Black and white photographs are included to demonstrate normal spermatozoa and spermatozoa with a variety of defects. There is a coloured photograph of normal spermatazoa stained with Papanicolaou stain which I did not think showed the red colour of the midpiece very well.

The tests for antispermatozoa antibodies include the Immunobead and IgG MAR tests.

Brief mention is made of biochemical tests including citric acid, zinc, fructose, acid phosphotase and a glucosidase.

Section 3 is about spermatozoa – cervical mucus interaction and would apply to specialist andrology laboratories rather than routine clinical laboratories. It inclues tests to evaluate cervical mucus and the examination of post coital specimens to determine the number of active spermatozoa, their behaviour and survival.

Section 4 is about spermatozoa preparation techniques for therapeutic reasons.

Section 5 is on the Quality Control with some suggestions for internal QC schemes.

The appendix makes up half the book and includes a table of normal values, safety guidelines plus the methods for the tests mentioned in the above sections.

As I have not read any textbooks on this topic but have used the RCPA broadsheet No. 20, 1979, titled "Semen Analysis", I have nothing to compare it with. However it is a concise book of 107 pages which I have found useful for standardising the methods in our laboratory which include the microscopic and macroscopic examination of semen. It may be too concise for specialist laboratories performing a wider variety of tests. It cost $102 and was purchased from Medical Books, Wellington.

Clinical Microbiology Procedures Handbook
Editor in Chief: Henry Isenberg. Publisher: ASM Press. Price $US240.00. Reviewed by: David Riley

This Handbook consists of two substantial ring binders containing nearly two thousand five hundred pages covering many procedures used in medical microbiology laboratories.

The stated purpose is to provide: "a 'cookbook' that provides step by step descriptions for the numerous methods that personnel at the bench must use."

Sections included are; Aerobic Bacteriology, Anaerobic Bacteriology, Mycobacteriology, Aerobic Actinomycetes,

Antimicrobial Susceptibility testing, Mycology, Parasitology, Viruses, Rickettsiae, Chlamydiae and Mycoplasmas, Immunology, Molecular Biology, Epidemiology and Infection Control, Instrument Maintenance and Quality Control, Laboratory Records and Water Quality, Biohazards and Safety.

Each procedure is detailed under section headings such as Principle, Specimens, Materials, Procedure, Results, Reporting Results, References and Supplemental Reading. Many excellent tables and diagrams are included, the sections on Instrument Maintenance and Quality Control are especially detailed and include practical information difficult to find elsewhere.

This handbook is highly recommended for any medical microbiology laboratory as an adjunct to standard textbooks and a valuable aid when preparing procedure manuals. The main drawback is the size of the two folders and the fragility of loose leaf pages bound only with ring binders.

Haematology

Special Interest Group

Convenor: Ross Anderson
Contact Address: c/- Diagnostic Laboratory, Box 5728, Auckland
Phone: (09) 357-4100
Fax: (09) 366-0642

The re-structuring of the Health Service on the Auckland Central Crown Health Enterprise (C.H.E.) site has precipitated a change in Haematology Special Interest Group (H.S.I.G.) office bearers.

Anne Cooke is now the Quality Officer for Auckland Health Care Laboratory Services. Alan Johns is the Operations Manager for the Auckland Health Care Laboratory Services. Rennie Dix is the Customer Services Manager for Auckland Health Care Laboratory Services.

Anne was formally the Haematology Tutor at the Auckland School of Medical Laboratory Technology. She has done sterling work as secretary of H.S.I.G. over the years and as Publicity Officer for H.S.I.G. Anne's input into matters relating to Education and Training over the years has been invaluable.

Alan has been a very valuable member of the H.S.I.G. Group, contributing to and participating in many of the seminars. He was responsible in particular for the success of the Haematology section at the South Pacific Congress held at the Aotea Centre in 1991.

Rennie has been the Treasurer of H.S.I.G. His assiduous attention to the fiscal policies of H.S.I.G., particularly when organising the very successful H.S.I.G. seminars over the past eight years has earned him the title of 'Mister Money Bags' amongst his colleagues.

To Anne, Alan and Rennie we say thank you for your contributions to H.S.I.G. over the years and wish you well in your new career directions.

The new office bearers:

Secretary:	David Underwood, North Shore Hospital.
Treasurer:	Horst Stunzer, Medical Laboratory.
Publicity:	Marilyn Eales, Middlemore Hospital.
Chairperson:	Ross Anderson, Diagnostic Laboratory (no change).
Committee Members:	Kathryn Schollum and Cindy Lincoln.

Cindy has been responsible for the H.S.I.G. Blood Film Survey over the past few years and has been co-opted onto the Committee. Other members will be co-opted as appropriate.

H.S.I.G. Seminar 1994

This year the H.S.I.G. Seminar will be run in Hamilton prior to the annual conference on Tuesday 30 August. The subject is to be 'Emergency Haematology'. Robin Alan is spear-heading the organisation of this seminar. Details of the programme are available in the conference section of this journal. Separate flyers will be distributed to Charge Technologists.

Technicon H3 RTX

Medlab Auckland has just installed two Technicon H3 RTX instruments. Medlab is the first New Zealand Laboratory to install these instruments which do reticulocytes as well as Full Blood Counts. Currently the reticulocyte mode is being evaluated. We look forward to hearing results of this evaluation. Felicity Ferret tells H.S.I.G. that Diagnostic Laboratory is installing an H3 RTX system as well. Watch this space!!

Degree Students

Tutors from Massey University (Dr Mary Knulsen and Mr Chris Kendrick) and Otago University (Professor Colin Watts) have been visiting students on-site in their various placements. Evaluation of the course after the first year of placements will be interesting and informative.

Immunology

Special Interest Group

Convenor: Gillian McLeay
Laboratory Training Centre,
Auckland Regional Blood Centre,
2-6 Park Avenue, Grafton
Auckland

NZIMLS ANNUAL SCIENTIFIC MEETING

This year is a first for ISIG. The two disciplines represented by our Special Interest Group are being given separate billings where possible, on the scientific program.

The Virology workshop (Rapid Diagnostic Testing) and first part of the Virology scientific program will be held on Thursday 1 September; the Immunology program will be on Friday 2 September.

There may be some conflict of interests on Friday when some forums will run concurrently. At the time of writing this article final details of the program were not confirmed. However, the scientific committee has done a grand job. There is must shared interest between the two disciplines and the program schedule will provide virologists and immunologists the opportunity to attend the majority of both programs.

Programs and presenters

Dr Eric Gowans, a hepatitis specialist from Queensland, is the guest speaker for the Virology forum on Thursday 31 August and will be talking about the ongoing problem of conflicting results in Hepatitis testing. His other topics include Hepatitis C in donor testing.

Other speakers are Dr Bryan Shroeder from Auckland (Hepatitis B) and Rachel Jenkins (Herpes simplex and another old faithful – rubella) from Dunedin. Virology, as one of the smaller disciplines, is to be congratulated on turning on such a full program.

Friday's Immunology program is right up to the minute with recent advances in DNA technology and nephelometry (the latter technique having replaced so many of the varied techniques of former years), and those perennial favourite topics – autoantibodies and autoimmune disorders.

There are three speakers from overseas. Dr Susan Tomasic-Allen's (Abbott Diagnostics, USA) topic is the Ligand Chain Reaction (LCR), DR Ricardo de Oliveira (Vice President R&D Hemagen Diagnostics USA), a Clinical Pathologist by profession, will be speaking on autoantibodies to intracellular antigens and Ian Edwards (Hoechst Australia) an ex-pat Kiwi, will be speaking about a new nephelometer and recent advances in that technology.

Nearer to home, Dr Patricia Stapleton (Technical Manager DNA Diagnostics, Auckland) will talk about paternity analysis using DNA profiling, Dr Allan Doube (Consultant Rheumatologist, Waikato Hospital), the clinical relevance of autoantibody tests, and last but not least, Joanne MacDonald (an RMLT from Christchurch Hospital) will discuss 'SLE and a case of Atypical Pneumonia".

Joanne's paper was voted best presentation at the North Island Seminar in April; the prize for best paper (sponsored by the organising committee of the conference scientific program) was a free registration to conference and an invitation to present the paper in the Immunology program. All SIGs were made a similar offer.

Programs and abstracts published in the June edition of the *ISIG Network News*.

ISIG LUNCH AND AGM

The traditional *ISIG* lunch and Annual Gathering of Members (the letters of which, coincidentally, are the same as those for annual general meeting, but do not reflect the formality of the latter) will be held at 1230 on Friday at the "Roundabout Cafe", a mere 200 metre walk from the conference venue. Please advise Tim Taylor, Medlab Hamilton (Ph. 07-838-0599, fax. 07-834-0758) of numbers wishing to attend.

It will be an opportunity to welcome the new *ISIG* convenor and committee, deal with a couple of items of business, thank our hosts and enjoy the hospitality of the Waikato branch of *ISIG*. The Treasurer will ensure the ISIG chequebook is on hand to pay the bill.

A brief meeting of *ISIG* members will be held early on 30 August (time to be advised) to confirm the convenor and committee. This is necessary this year, as the newly-elected convenor will be attending the Council/SIG Convenors' Annual Meeting to be held probably later the same day. Please attend to ensure democracy is served.

* * *

ANZAC WEEKEND GATHERING AT TAUPO YACHT CLUB

The *ISIG* North Island Seminar on Saturday 25th and the Coulter Users' Flow-cytometry Meeting on Sunday 26th April once again proved to be a stimulating and convivial weekend for *Network* members and those who practice the art of flowcytometry. The weather may not have been quite as brilliant as last year – it rained on Saturday, but cleared up Sunday – but holding the event over the holiday weekend took some of the pressure off those who had travelled long distances.

We were pleased to welcome Dennis Reilly (NZIMLS President) who not only opened the seminar but presented a paper also, Graeme Chapman (Coulter, Sydney) who spoke at the seminar and hosted the Flowcytometry meeting, Greg Murrell (National Sales Manager, Coulter, Sydney), Elaine Scrugham (Coulter, NZ) and Professor John Clarke from Massey University, who spoke about the MLS courses at Massey. (Graeme and Professor John are members of the ISIG Network.)

IMMUNOLOGY SEMINAR

The Saturday program, which commenced at 1230 was divided into four sessions. A general forum, a clinical forum of two sessions and a short business forum.

WELCOME TO GUESTS AND DELEGATES

Dennis Reilly welcomed everybody and commented on the continuing success of the Special Interest Groups.

GENERAL FORUM

* BMLS Course

Professor Clarke outlined the BMLS course, selection of students, and course content for the Virology/Immunology pathway for each year. David Haines (Auckland) and Ray Cursons (Hamilton) will be moderators for the final examination. He named and thanks those ISIG members who had assisted with the development of the course.

The course will be inspected by the AIMLS for reciprocal registration with Australian qualifications. Regarding MLTB

registration for Massey students, Professor Clarke stated that registration either at graduation or following an internship was acceptable to the University.

*** Diploma in MLS**
Professor Clarke described the course which is designed for technologists, educated and trained under another system, who wish to gain a further qualification. People can take the existing fourth year courses.

*** The Birth of a New Department**
Dennis Reilly has introduced a new term into the MLS vocabulary. Biochemistry and Immunology at Diagnostic Laboratory in Auckland have combined to become "BiochImm". Reasons are that changes in technology are reducing the number of instruments required and it makes sense to share them.

Goals of this merger are to flatten management structure making it more responsive to staff, promote adaptability and innovation, low levels of formalisation, high emphasis on job satisfaction, amplify job skills and knowledge, break down barriers.

Amalgamation has been successful. Laboratory no longer compartmentalised; changes give opportunity to learn. Increase in productivity _ more tests, more staff – are partly due to amalgamation. No redundancies. New training bench set up – staff rostered to learn new techniques.

*** Monitoring Staff Health in the Laboratory**
Susan Duncan (H&S Representative at Diagnostic Laboratory, Wanganui) introduced this discussion topic, looking at monitoring of staff before and after accidents or incidents.

The introduction of legislation makes it very important to have guidelines and to have pre-employment checking available also. She suggested doing baseline blood tests at beginning of employment, but admitted there could be problems.

To preserve confidentality, taking these coded samples, or where counselling is required (eg. HIV) employee could go to GP of choice and employer would pay 2/3 of fee. Results to employer only with consent of employee.

CLINICAL FORUM (1st Session)
*** Identification of HIV-II in a NZ Resident**
Dr Bryan Schroeder (Invited Speaker) described a recent case study where HIV-II infection was diagnosed. HIV-II is mostly concentrated in West Africa, but spreading into Asian heterosexual group. Some serological cross reactivity with HIV-I. Important to develop specific PCR probe.

*** Testing for Giardia Antibodies**
Professor CLarke suggested that serological diagnosis by EIA not a practical proposition; too much background to determine clinical infection as opposed to residual antibodies for IgG and IgM, or even IgA.

Discussed different types of giardia, water testing and human and animal infection – 20-30% cats and dogs have giardia in faeces. PCRs the way to identify different types. Graeme Chapman suggests flowcytometry as an alternative.

*** CMV – Alive and Kicking**
Lilian Martin (Hutt Valley Health) gave an interesting talk on the laboratory diagnosis of adult and paediatric CMV infection. Showed lists of CMV results plus others (eg. HBV, EBV, Rubella, Toxoplasma).

*** Allergy Testing**
Rodger Linton (Health South Canterbury) introduced this topic. Standardised guidelines for performing and interpreting skin tests needed for laboratories offering this diagnostic service. David Haines (Auckland), Joanne MacDonald (Christchurch) and Jenni Hillas (Immunotherapy Specialist, Ebos) all with first hand practical experience contributed to the discussion. Jenni advised that legal aspects of using "home made" products should be checked out.

CLINICAL FORUM (2nd Session)

*** Brucella Case Study**
Michael Crowther (Diagnostic Laboratory, Auckland) reported on a case of Brucella suis. Michael's slides were particularly colourful and graphic.

*** Yersinia-triggered Arthritis**
Rubee Yee (Hutt Valley Health) presented this case study which she presented also to the Microbiology SIG seminar in March; just as relevant for immunologists, it prompted some good discussion.

*** Heterophile Negative Mononucleosis**
Darrell Monk (Diagnostic Laboratory, Wanganui) presented interesting cases where the Haematology Department had suggested a possible diagnosis of infectious mononucleosis on blood count and film. Negative heterophile antibody results necessitated testing a wide range of infective agents. Liver function tests were mostly abnormal. A CMV latex screen and captia (mostly laboratory-initiated) finally confirmed CMV infection. An interesting piece of immunological detective work.

SLE and the Case of Atypical Pneumonia
Joanne MacDonald (Canterbury Health Laboratories) described two cases she had encountered. He presentation, which included excellent slides, won her the prize for best paper for the day and will give her free registration to the NZIMLS Annual Scientific Meeting in August. Similar cases have been reported in Palmerston North also.

*** Sensitivity of Hep-2 to SSA**
Gerry Campbell (Medlab Wellington) compared the sensitivity of different Hep-2 slides (substrate for anti-nuclear antibody tests) for detecting the presence of the antibody to the SSA extractable nuclear antigen and advised on which brand gave the best results.

*** DNA Antibody Testing**
Michael Crowther (Diagnostic Laboratory, Auckland) presented his second paper of the day as a discussion topic. His laboratory compared a variety of methods (Kodak RIA, EIA methods with the IFA (crithidia substrate) and will be using the Sanofi EIA kit.

*** Flowcytometry**
Dr Graeme Chapman (Coulter, Sydney) stepped into the gap in the program left by Maurice Roberts (ARBC, Auckland) who was unable at the last minute to attend to present his paper, "Transplantation Records". Graeme gave a brief review of flowcytometry and unveiled Coulter's latest model of the XL Flowcytometer.

BUSINESS FORUM
The business forum consisted of an update on ISIG issues and activities, and a Conference Preview presented by Tim Taylor (Medlab Hamilton) who is on the scientific organising committee.

SOCIAL FUNCTION
Saturday's program concluded with a Happy Hour followed by a Buffet Dinner, also at the Taupo Yacht Club. Joanne MacDonald was named winner of the best paper for the day.

COULTER USERS' FLOWCYTOMETRY MEETING & WORKSHOP
The Sunday program sponsored by Coulter Electronics (NZ) Ltd provided an interesting and constructive morning for those who utilise flowcytometry as a diagnostic tool. Hosted by Graeme Chapman, a great deal of thought and preparation is involved in setting up this meeting and workshop which was custom-made for the eight participants. These included ISIG members and folk from different disciplines, both within and outside medical laboratory science.

NEW PRODUCTS AND SERVICES

INTRODUCING THE ABL5 FROM RADIOMETER

Radiometer Copenhagen has just released its latest blood gas analyser, the ABL5, incorporating the essentials of blood gas for the smaller throughput departments.

The ABL5 provides essential blood gas and acid-base information from an 85µL whole blood sample measuring pH, pCO_2 and pO_2 and then calculates sO_2, HCO_3, tO_2, $AaDpO_2$, SBC, ABE, SBE and tCO_2, all in 60 seconds.

Designed to be extremely straightforward, the operator simply positions the sample collecting device against the probe and presses the aspiration button. Operation is menu driven.

The ABL5 is a true STAT analyser where calibrations can be interrupted at any time for emergencies. A standby mode is incorporated as standard and is ready in a few minutes when brought out of standby. The ABL5 is compact and lightweight making it easily transferred and shared between departments.

Little maintenance is required on the ABL5, limited to checking the solutions and gas pressure. The ABL5 uses the unique "Click and Go" remembraning of all electrodes and is most cost effective compared with disposable electrodes.

The ABL5 is the perfect main analyser for departments with a smaller throughput of blood gas samples or as a second analyser for larger hospitals.

For further information relating to the Radiometer ABL5 blood gas analyser, please contact Radiometer Pacific Ltd on (09) 573 1110. Radiometer Pacific Ltd, Unit A, 10-20 Sylvia Park Road, Penrose, Auckland.

Graeme described the new, updated features of the XL Flowcytometer – ie. fibreoptic cables for instant information to and from work station, laser printer, upgrading to four colours, a computer (plus mouse) which will be upgraded continually, and which can be networked, to name but a few. (Phenotyping "on auto" in 19 minutes will be a real timesaver.)

He also outlined the improvements found in the Coulter Elite ESP (a high performance cell sorter). The optics have been improved, especially in the red spectrum, there is improved sensitivity, a higher flow rate and considerable development in the lasers with a range of options and me flexibility.

The workshop sessions consisted of trouble-shooting. Many of the participants are the only ones in their laboratories with sufficient knowledge and skill to resolve problems encountered with the operation of this advanced technology. Sometimes even this is not enough.

The workshop is a unique opportunity to share experience and gain advice and support. Participants had sent in the topics they wished discussed prior to the meeting. Graeme Chapman's vast technical knowledge and practical experience provides a great resource for New Zealanders in this field. To most questions, satisfactory answers were provided, which will enable the resolution of problems until a similar meeting is held next year.

The meeting concluded with a lunch provided by Coulter Electronics (NZ) Ltd.

THE FINAL BOW

This is the 13th ISIG update I have written for the Journal and the last I shall write as convenor, although I may contribute something from time to time, if invited to do so.

I wish the new convenor and incoming committee all the best for the future and hope, under their guidance, ISIG will continue to grow and develop.

Gillian McLeay
ISIG Convenor (1991-1994)

Transfusion Science

Special Interest Group

Convenor: David Wilson
Contact Address: Sheryl Khull, Transfusion Medicine Dept, Palmerston North Hospital, Private Bag, Palmerston North
Current members: Allison Dent, Auckland Blood Centre
Sue Baird, Blood Bank, Lakeland Hospital, Rotorua
Roger Austin, Blood Bank, Taranaki Base Hospital, New Plymouth
Marie Willson, Blood Bank, Teraiwhiti Hospital Gisborne
Kevin McLoughlin, Transfusion Medicine, Christchurch Hospital
Les Milligan, Blood Bank, Dunedin Hospital

HELP!?

The list of current members of the Transfusion Science Special Interest Group is above. Many of these people have been on the TSSIG since soon after its inception in 1989. (For a history of the Special Interest Groups, see the May 1994 edition of NZJ Med Lab Science).

The NZIMLS has suggested that SIG members should serve three years – partly to ensure some continuity, and partly to ensure a continual influx of fresh members with fresh ideas and enthusiasm.

This is where **you** come in.

The Transfusion Science Special Interest Group needs some fresh ideas and enthusiasm. Have you got some? I know everyone is busy with their own work at the moment (is that an understatement for you too?) but couldn't you manage to give just a little time and effort back to the profession that nurtured you through your training and into the position where you are so valued and in demand?

The TSSIG will be meeting in Hamilton during Conference week. If you're planning to be at Conference, enjoying the benefits of sharing the knowledge and experience of others and updating your skills through workshops organised by the NZIMLS, why don't you contact one of the TSSIG members and offer to lend a hand? You'd be surprised at how much benefit you can get back from such altruistic works.

And if you're not planning to be at Conference this year, for whatever reason, (I know someone has to stay home and hold the fort) you can still be involved and your input gladly welcomed. Just contact one of the members and make the offer.

Thank you.

P.S. Wouldn't it be lovely if everyone working in Transfusion Science in New Zealand wanted to contributed something? Couldn't we really go places then!

WHATEVER WILL THEY THINK OF NEXT?

"Instant Platelets – Just Add Water"?

Researchers at the University of North Carolina announced at an American Society of Haematology meeting late last year that they have developed a process for fixing, drying and rehydrating platelets for transfusion. The platelets appear to function well in animal models.

That could make life easier!

THE GEL TEST

Marie Willson sent in the following summary of an article which appeared in Laboratory Medicine in February.

The gel test, as developed by Lapierre, is an innovative approach to blood group serology. This technology addresses the issue of standardisation and incorporates sensitivity, specificity and efficiency; it is accessible to laboratories of all sizes and levels of expertise. By employing gels premixed with reagents, specific volumes, and a no-wash antiglobulin

test that eliminates resuspension of red cell buttons, the gel test reduces the variation inherent in conventional techniques. Applicable to a broad range of tests routinely performed in the blood bank, such as antibody screening, antigen typing and crossmatching, the gel test procedures are easy to perform and yield clear cut end points that are stable, plus they can be reviewed at a later time. Applications for the technology reach beyond the routine and potentially into other areas of diagnostic testing.

The gel test is one of the new technologies which will be available at the TSSIG workshop at Conference.

NICE OZZIES

New Zealand Transfusion Scientists are invited to the Australian NICE Weekend. Actually the Australian one is the original – it has been going for five years longer than the New Zealand version, and we pinched the name from them.

Numbers are restricted, so make a decision as soon as possible. The organisations will do their best to fit any kiwis in. Instructions and help in getting to Wodonga are available.

The application form is printed elsewhere in this Journal. (see p.)

TRANSFUSION MEDICINE AUDIO UPDATES

A continuing education programme presented on audio tape available through the Transfusion Science Special Interest Group.

LATEST TOPICS!

0893 Red Cell Antibody Case Studies: Beginning to Intermediate Level
0993 Red Cell Antibody Case Studies: Advanced Level
1093 Computerisation of the Transfusion Service: A General Overview
1293 Case Studies in Transfusion Medicine
0294 Current Issues in Autologous Blood Donation and Transfusion

To Order:
Send a cheque or company purchase order for $6 per topic to Sheryl Khull, Transfusion Medicine Dept, Palmerston North Hospital. (Non-members of NZIMLS add $2 surcharge)

LEUCOCYTE DEPLETION WHEN? WHERE? HOW?

K McGRATH, DIRECTOR OF DIAGNOSTIC HAEMATOLOGY, ROYAL MELBOURNE HOSPITAL

WHY?

The donor white cells present in all cellular blood components prepared by the standard techniques are known to cause a wide variety of side effects when transfused into patients requiring red cell or platelet transfusions. The recognised consequences of white cell transfusion include stem cell engraftment, transmission of intracellular organisms such as cytomegalovirus, EBV and HTVL-1, stimulation of HLA and granulocyte specific antibodies, and a generalised immunosuppression. These complications are manifested clinically as graft vs host disease, post transfusion viral infection, febrile reactions, refractoriness to platelet transfusions, improved survival of transplanted kidneys, reduced engraftment of transplanted bone marrow and possibly increased incidence of cancer recurrence and post operative infection. More recently there has been a suggestion that white cell contamination of blood products may prolong haemopoietic recovery post chemotherapy in acute myeloid leukaemia, and accelerate the rate of progression of HIV infection.

WHY NOT?

There are few identifiable benefits to the retention of white cells in transfused blood products apart from the extension of kidney transplant survival. The use of leucodepleted blood components is therefore being widely recommended and practiced. Indeed, the question of whether all products should be leucodepleted is now being raised.

The major argument against leucodepletion is the cost of white cell removal. Leucocyte depletion adds a signifcant cost to each transfusion episode. Buffy coat removal costs approximately $2 per unit whle second and third generation filters cost between $15-$60 per RCC or platelet transfusion. Thus leucodepletion must be shown to be cost effective. This means that their influence on patient clinical outcome must be shown to be of sufficient benefit to warrant the cost of treating all eligible patients within defined categories.

HOW?

The simplest form of leucodepletion is the removal of the buffy coat from a unit of blood following centrifugation. This results in a one log or 60-70% reduction in white cells. The spin-cool-filter techniques using microaggregate filtration also results in approximately a one log reduction.

To achieve a two to three log or ">99% reduction in red cell or platelet concentrates, filtration through filters composed of cotton wool, cellulose acetate or polyester fibres, either native or surface modified by chemical or ionisation treatment is required. Different filters are required for red cell and platelet concentrates. Filters used for RCC generally also remove platelets and can remove up to 25% of red cells. In pooled platelet concentrates a three log reduction to >5x101/46 leucocytes per 3x101/411/411/4 plateletes can be achieved by third generation filters specifically designed to remove leucocytes with minimal removal of platelets.

These newer generation filters can be used in the central transfusion service, in the hospital blood bank or at the bedside. Under controlled conditions they are effective and efficient. At the bedside, the conditions for use are not as readily controlled and the efficiency of the filters has not been properly validated. As bedside usage has increased exponentially, it is now essential that appropriate guidelines for their use be established and validated in each institution. Given the low residual white count in filtered products, newer methods of white cell enumeration such as flow cytometry may be necessary to ensure accurate counting of cells.

Newer filters are being developed which can result in up to six log white cell reduction.

PROVEN INDICATIONS FOR LEUCODEPLETION

The indications for which leucodepletion can be currently shown as having well documented clinical benefit are listed below.

Febrile Reactions to Red Cell Transfusions

These reactions are due to presensitisation to HLA or neutrophil specific antigens.

There is clear evidence in well designed studies that leucodepletion will prevent febrile reactions in patients receiving red cell containing products. Patients should have had at least two consecutive febrile reactions before leucodepletion is employed. The level of leucodepletion can be varied. Many patients will have no further reactions with a one log reduction in whit cells achieved by buffy coat depletion. Other patients will tolerate buffy coat poor products for 6-12 months before febrile reaction recur.

Once reactions recur, virtually all can be prevented by third generation filters removing three log of white cells (residual cells <5x10¼6). Further modalities include washed filtered or frozen and thawed red cell concentrates, and these are usually effective

when reactions occur with filtered red cell concentrates.

One question that remains unclear is whether leucodepletion should be used to prevent the development of febrile reactions in patients with diseases such as thalassemia major, requiring regular transfusion. Not all these patients develop febrile reactions and the reactions can be effectively alleviated by subsequent leucodepletion. Thus there seems no good argument to prevent the onset of febrile reactions. Leucodepletion in graded steps (buffy coat poor, filtered, washed/filtered and frozen) should be introduced sequentially once regular reactions occur.

Prevention of Presensitisation of Aplastic Anaemia

Patients with aplastic anaemia who are possible marrow transplant recipients should receive cellular blood products that are leucodepleted to <5x10¼6 WCC in order to maximise the likelihood of marrow engraftment.

Prevention of CMV Transmission

There is well documented evidence that removal of leucocytes from CMV containing cellular blood components to a level of <5x106 will prevent the transmission of CMV.

The question to be considered, however, is whether this is a cost effective approach when testing for CMV antibodies can identify CMV negative blood products. Viral antibody testing is considerably cheaper and therefore the method of choice for selecting CMV negative blood products. Filtration for this purpose should be restricted to the transfusion of cellular products to immunosuppressed patients who are CMV negative or whose status is unknown and for whom known CMV antibody negative products are unavailable.

Intrauterine Transfusions

Although there are no definitive clinical studies demonstrating a clinical benefit of filtration of blood used for intrauterine transfusions, nevertheless, the prevention of further immunosuppression in the foetus with an immature immune system, prevention of viral transfmission and prevention of maternal alloimmunisation to neutrophils would appear to be warranted in this situation by analogy with comparable adult immunosuppressed patients. The WCC count should be reduced to <5x10¼6/unit.

POSSIBLE INDICATIONS FOR LEUCODEPLETION – CLINICAL BENEFIT YET TO BE ESTABLISHED

There are a number of indications for which the evidence of a clear benefit to patient management has not yet been demonstrated. There may be well documented changes in laboratory based parameters but good prospective studies showing an improvement in patient morbidity or mortality are needed.

Febrile Reactions to Platelet Transfusions

There are few studies reporting the efficacy of filtration for this purpose with the third generation platelet specific filters. One report by Mangano et al demonstrates that filtration prevents only a relatively small proportion of febrile reactions to platelet transfusion and our own personal experience would support this. The clear difference between filtration of RCC and platelets in this setting may relate to the temperature of storage and the continued metabolism of white cells at room temperature during platelet storage with release into the plasma of vasoactive substances or cytokines which are responsible for the febrile response. The findings reported by Mangano et al need confirmation in larger prospective studies before the value of filtration at the time of transfusion of platelet concentrates for the prevention of febrile reactions can be fully assessed.

Prevention of Alloimmunisation to Leucocyte and Platelets

There are many studies which clearly demonstrate that the depletion of leucocytes to <5x10¼6 in RCC and platelet concentrates is very effective in preventing the development of HLA antibodies post transfusion. HLA antibodies are the major cause of alloimmunisation and the poor platelet increments seen in patients who are alloimmunised. Many authors therefore conclude that prevention of alloimmunisation will prevent poor platelet increments occurring in patients undergoing leukaemic induction therapy or bone marrow transplantation. This latter clinical benefit from filtration has not been demonstrated in prospective clinical studies. It is well known that there are many other non-immune mediated causes of poor platelet increments including infection, hypersplenism, DIC, and the tissue damage associated with marrow transplant preparative regimens. Further many patients develop HLA antibodies towards the recovery phase of induction therapy and the antibodies will often have disappeared prior to subsequent courses of chemotherapy. There are other modalities for overcoming alloimmunisation viz:- platelet crossmatching or selection of HLA donors from typed cytapheresis panels. Thus a prospective study assessing the clinical and cost benefit of preventing alloimmunisation by filtration, as compared to the provision of compatible platelet by matching donors needs to be performed before the widespread use of filtration for this purpose can be recommended.

The use of filtration to prevent presensitisation of potential renal transplant recipients to HLA antigens however may be a more legitimate indication. Again a proper prospective clinical trial is needed to document the clinical benefits of this approach.

Prevention of the Immunosuppressive Consequence of Transfusion

One of the most controversial areas of transfusion medicine relates to the immunosuppressive effects of cellular transfusions. Transfusion of whole blood or red cell concentrates pretransplantation significantly improved the survival of renal transplants. This effect was stronger with white cell containing products than with washed or frozen red cells. Laboratory evidence of reduced immune function following transfusion include decreased cytokine production, reduced MLR responses, diminished responses to mitogens and soluble antigens, decreased helper T cell and natural killer cell levels.

Increased cancer recurrence rates have been reported for patients receiving transfusions at the time of surgery for cancer of the colon. All studies except one to date have been retrospective and the association with transfusion has been variable although largely in favour of the association. The link is less strong with other types of cancer. It is difficult however to separate the effects of tumour stage, extent of surgery, pre-operative anaemia and age from the transfusion effect. This association has not been confirmed in the single prospective study comparing homologous and autologous blood. It is therefore difficult to recommend filtration for this purpose. The same situation is true of the increasing number of studies demonstrating an increased incidence of post operative infection in patients transfused at the time of surgery. Again the impact of transfusion is difficult to separate from the length and complexity of the surgery. A prospective study with homologous and autologous blood products is needed to clarify the role of transfusion. Thus it is currently difficult to justify the major expenditure involved infiltration of all leucocyte containing products in patients undergoing major cancer or other surgery to prevent possible increased risk of cancer recurrence or infection. The same assessment can be made regarding the use of leucodepleted products in neonates of adequate birth weight and maturity where filtration is sometimes used to prevent immunosuppression.

Transfusion of Cellular Products to HIV Infected Patients

There is some preliminary evidence to suggest that white cell containing blood products may activate HIV1 replication and upregulate HIV1 expression when cocultured with infected peripheral blood mononuclear cells. There is however only one in vitro study demonstrating these findings. Thus routine filtration for cellular products in HIV infected patients cannot yet be recommended.

Role In Recovery of Cytopenias In AML Induction Therapy

There is preliminary but tantalising data to suggest that whitecell containing blood products may delay the recovery of neutropenia and thrombocytopenia in patients undergoing sequential courses of chemotherapy for AML induction and consolidation. This data is from one single study and requires confirmation.

INAPPROPRIATE USES OF LEUCODEPLETION

Prevention of transfusion related graft versus host disease is best achieved by gamma irradiation. Transmission of hepatitis B or CHTLV and HIV cannot be prevented by leucodepletion. The transfusion related acute lung injury (TRALI) cannot be prevented by leucodepletion.

FUTURE EVALUATION

The role of leucodepletion is a complex one and its ultimate place in transfusion is yet to be determined. Health care professionals now have a responsibility to ensure that new modalities of treatment produce a clearly definable benefit to the patients in whom they are used. Cost is but one factor that must be assessed, but it is no longer appropriate to recommend new and expensive techniques such as leucodepletion without well documented evidence of its efficacy.

This approach has been recently affirmed by the Royal College of Physicians of Edinburgh who held a consensus conference on leucodepletion. The resulting guidelines were a major reference for the article and as such the recommendations included in this article are very similar.

There is likely to be a considerable amount of information published on this topic over the next few years and the role of leucodepletion will need to be regularly reassessed in the light of results from the relevant clinical trials.

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John Ruskin 19th Century English writer and social reformer.

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Oh, give me your pity, I'm on a committee
Which means that from morning to night
We attend, and amend, and contend, and defend
Without a conclusion in sight
We confer and concur, we defer and demur
And re-iterate all of our thoughts
We revise the agenda with frequent addenda and consider loads of reports
We compose and propose,
we suppose and oppose
And the points of procedure are fun!
But though various notions are brought up as motions there's terribly little gets done.
We resolve and absolve, but never dissolve
Since it's out of the question for us
What a shattering pity to end our committee
Where else could we make such a fuss??!!

Biochemistry

Special Interest Group

Convenor: Allison Buchanan
Contact Address: Clinical Chemistry Department
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Clinical Chemistry Competition

We had several entries in our competition. Two people have agreed to present at the Hamilton conference on September 1st thus gaining free registration and a prize. Two others have agreed to write a paper on their chosen topic, and these will be submitted to the journal for publication. Prizes will also be offered to these people.

Our thanks to all those who showed interest and we look forward to hearing your presentation at conference or reading your paper in a future journal.

Keep up the good work.

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Send your completed application to the NZIMLS Executive Officer, PO Box 3270, Christchurch to be received no later than 5pm, 31st March 1995.

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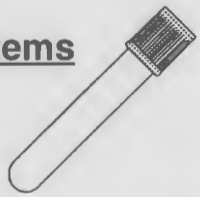

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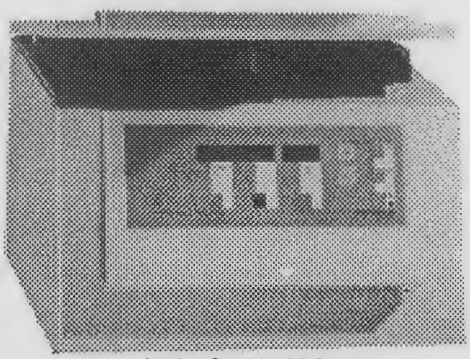

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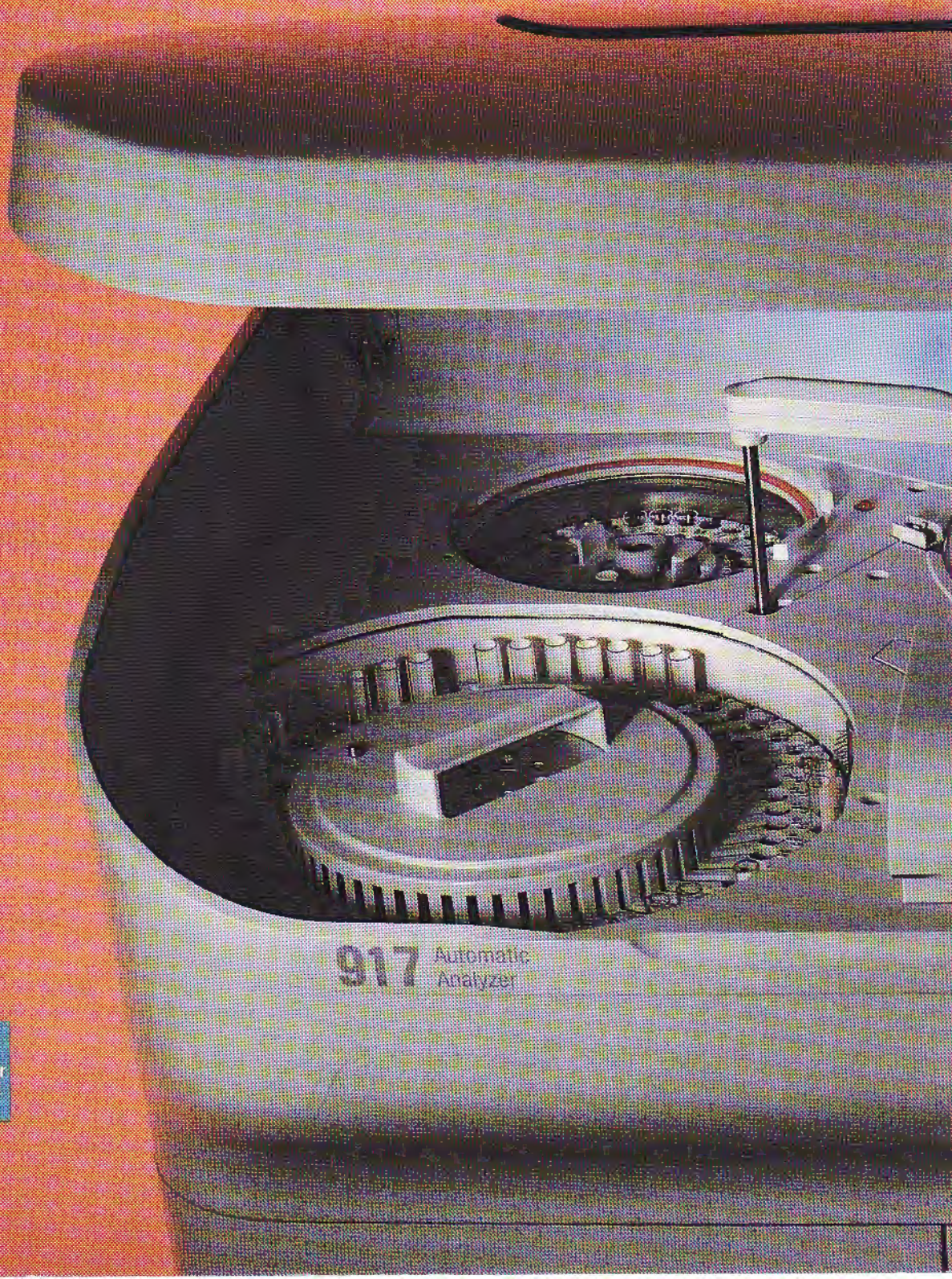

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