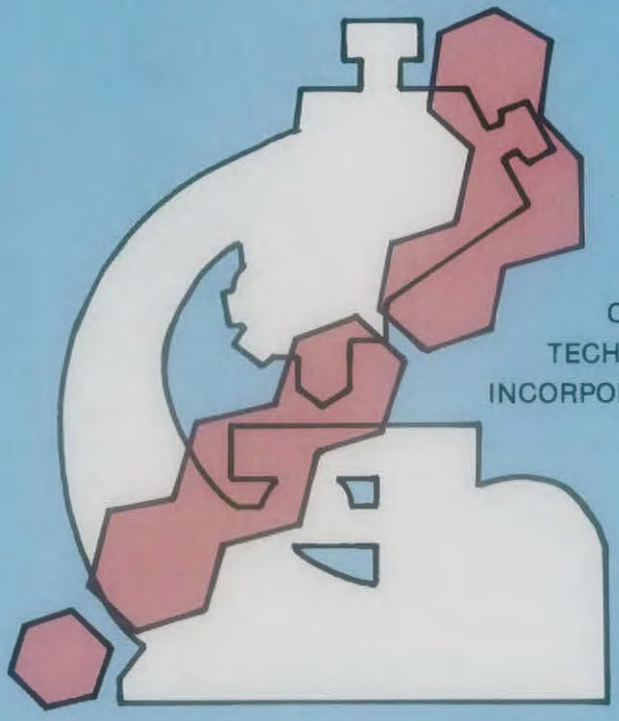
VOL 36 No. 5 December 1982

ISSN 0028-8349

NEW ZEALAND JOURNAL

OF

MEDICAL LABORATORY TECHNOLOGY



OFFICIAL PUBLICATION OF THE
NEW ZEALAND INSTITUTE
OF MEDICAL LABORATORY
TECHNOLOGY
INCORPORATED

ALL ASPECTS OF MEDICAL LABORATORY SCIENCE

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THE NEW ZEALAND JOURNAL OF
Medical Laboratory Technology

Vol. 36 No. 5 ISSN 0028-8349 December 1982

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Subscriptions to the Journal for non members requiring delivery in New Zealand is $NZ18.00 for 6 issues surface mail paid. Single issues are $NZ3.50 surface mail paid.

Subscription to the Journal for non-members requiring delivery overseas is $NZ18.00 for 6 issues plus $NZ4.20 surface mail paid. All subscriptions except for single issues are due in February.

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Intending contributors should submit their material to the Editor, P.O. Box 6168, Dunedin, New Zealand. Acceptance is at the discretion of the Editor, and no undertaking is given that any article will be published in a particular issue. The copy deadline for each is the first of the month prior to the month of publication.

ADVERTISERS INQUIRIES

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DATES OF PUBLICATION

The months of publication for 1983 are February, April, June, August, October and December.

This Journal is abstracted by: Biological Abstracts, Cumulative Index Nursing and Allied Health Literature, Current Clinical Chemistry, Hospital Abstracts, Institut nautchnoi informatsli.

The Quality of Antibiotic Susceptibility Discs

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Abstract

Batches of the two New Zealand manufacturers', Alpha and Biolab, antibiotic susceptibility discs and of two imported brands, Difco and Mast, were tested for compliance with the WHO requirements for potency and nationally set limits for uniformity. Biolab and Difco discs, with overall pass rates of 75.0 percent and 88.2 percent respectively, were considered to be of acceptable quality. On the contrary the quality of the Alpha and Mast discs tested was poor, the overall pass rates being only 6.7 percent and 25.0 percent. This poor performance may in part have been due to the considerable difference in the thickness and perhaps composition of the paper that these two manufacturers use and that used to make the standard discs. An improvement in the standard of Alpha's discs is expected following changes in production procedures.

Introduction

Five years ago the National Health Institute first surveyed antibiotic susceptibility discs made by the two New Zealand manufacturers.[1] Only 24 percent of the 41 batches tested, 10 percent of one manufacturer's and 40 percent of the others, passed the United States Food and Drug Administration (FDA) regulations for antibiotic susceptibility discs.[2] These results were of concern to both the industry and the Department of Health. Considerable effort has been exerted during the last three years to produce a better quality disc, to agree upon specifications to be used in New Zealand and in particular to decide upon the paper that standard discs should be made of, a factor which as is discussed later is critical in the assessment of potency.

In 1977 the World Health Organization (WHO) published requirements for antibiotic susceptibility tests which include some specific details of the production and control of antibiotic susceptibility discs.[3] It was decided, with the agreement of the manufacturers, that these international rather than the national regulations of the USA, were the more appropriate specifications for the New Zealand manufactured discs to comply with. The WHO requirements state that the mean potency of a batch of discs shall be 75-135 percent of the label claim but leave the specification for uniformity, within batch variation, to the national control authority. A limit in the form of a maximum variation of 33 percent in the potencies of individual discs around the mean was set after preliminary work and discussion. The quality of the discs produced by the two New Zealand manufacturers, Alpha Biologicals Limited (Alpha) and Biological Laboratories Limited (Biolab), has now been reassessed by testing batches for compliance with these requirements. Not all discs manufactured were tested but rather most of those that are recommended to be used in routine susceptibility testing.[4] The USA manufactured Difco Laboratories (Difco) and British manufactured Mast Laboratories Limited (Mast) discs were included in the survey for comparison. Until recently all batches of discs manufactured in the USA, intended for the domestic market, were subject to FDA testing and certification. It was therefore expected that the standard of discs manufactured in the USA would be high.

Materials and Methods

TEST DISCS:

Alpha and Biolab discs were collected by public health pharmacists from the manufacturer or their agents. Difco and Mast discs were obtained by routine ordering from their New Zealand agents. Discs were stored at -20 °C in their original packaging except Difco discs. These were received as loose, unprotected cartridges, the agent having split 10-cartridge packs to obtain the two cartridges that we required of each type of disc. These cartridges were stored with desiccant. Biolab discs were also packed in cartridges, Alpha and Mast discs were in vials. Only one batch of each type of disc was tested except where the two cartridges or vials received were of different batches.

STANDARD DISCS:

Pure samples of antibiotic powders were obtained from local suppliers. Discs, 6.5 mm in diameter, of Schleicher and Schuell paper, grade 740-E (S & S paper), were supported on a wire mesh. 0.02 ml of antibiotic solutions of appropriate concentrations were applied to discs to obtain standard discs of three contents, 50, 100 and 200 percent of the label claim of the discs to be tested. Solvents used to make these solutions are shown in Table 1. Discs were dried for two hours at 37 °C in circulating air. Standard discs were also made from Alpha manufacturing paper. Alpha discs were assayed against both sets of standard discs.

ASSAY:

Test discs were assayed against standard discs using a large plate microbiological agar diffusion assay. The range of discs tested is shown in Table 1. Several criteria, a straight dose/response line of adequate slope, clear easy to read zone of inhibition edges and suitable zone sizes were considered when choosing the media, organism and inoculum for the assay of each antibiotic disc. Details of the media and assay organisms used are given in Table 1. Spore suspensions of the Bacillus species and vegetative cells, harvested from overnight cultures, of all other assay organisms were used. Sterile 317 mm square glass plates were placed on a level surface. A seeded overlay of 150 ml agar was poured over a 250 ml base layer. The poured plate was air dried for 30 minutes in a laminar flow cabinet.

Test discs were allowed to equilibrate to room temperature. Eight replicates of each of the three standard discs and eight discs from each test batch were placed in an 8 x 8 configuration on the plate. Up to five test batches could be tested on the one plate. The placement of all discs was randomized according to a latin square design. A prediffusion period of generally 30 minutes at room temperature was allowed before incubation at 37 °C for 16-18 hours.

ANALYSIS OF ASSAY:

Zones of inhibition diameters were read on a zone reader (Autodata Scientific Limited, England). Data was transformed if necessary. The mean potency, relative to the 100 percent standard disc, with associated 95 percent confidence limits (upper and lower fiducial limits) was estimated for each test batch.[6] Of the eight discs tested per batch the potencies of the discs that produced the smallest and the largest zones of inhibition were estimated and expressed as a percentage of the estimated mean potency to determine the uniformity of the batch. A gross analysis of variance for the whole plate and an analysis of variance for the three standard doses were incorporated into the programmed procedure to check the validity of the assay. A batch of discs was acceptable if its potency was between 75-135 percent (upper fiducial limit $\geq$ 75 percent, lower fiducial limit $\leq$ 135 percent) of the label claim

and the least potent and most potent of the discs sampled did not deviate from the mean potency by more than 33 percent.

Results and Discussion

Table 2 tabulates, for each manufacturer, the number of batches tested and the percentage that passed the specifications.

POTENCY:

Biolab and Difco discs achieved a satisfactory pass rate but only 40.0 percent of Alpha discs and 50.0 percent of Mast discs passed the specification for potency.

With Alpha's discs a substantial increase, 40.0 percent to 80.0 percent in the pass rate was achieved when these discs were assayed against standard discs made of the manufacturing paper. Six of the ten batches that appeared overpotent against standards made of S & S paper had acceptable potencies when assayed against standards made of the manufacturing paper and the potency estimates of all other batches, except methicillin, were also lowered.

These observations illustrate that the paper used for the standard discs is a crucial factor in the assay of antibiotic susceptibility discs. The higher potencies estimated when the discs were assayed against S & S paper were probably due to the antibiotic being released less readily from this paper than the considerably thinner manufacturing paper. It could be argued that in fairness standard discs should be made of the manufacturing paper. However, because of the different release properties of various papers, and indeed even different batches of the same paper,[7] discs from several manufacturers, labelled similarly, will very likely produce quite a range of zone of inhibition diameters when used under the same conditions. Such a situation is unacceptable especially if results of susceptibility tests are obtained by reference to interpretative tables such as in the Kirby-Bauer method. Therefore all discs labelled to contain, for example, 30 μg tetracycline, should "perform" like 30 μg tetracycline on a recognised standard paper. Schleicher and Schuell paper 740-E was chosen for use as the standard paper following guidance on this matter from the WHO. The suitability of an individual batch of this paper should be checked by comparing its performance with that of the FDA master lot.

The low pass rate observed for Mast discs was also probably due to an appreciable difference in the release properties of their paper and S & S paper.

All standard discs were 6.5 mm in diameter. Alpha discs, 8 mm in diameter, were the only test discs with a diameter appreciably different from the standards. The WHO requirements[3] state that standard discs should be of the same diameter as the test discs. The effect of the paper on the performance of a disc has just been discussed; similarly the diameter of the disc affects its performance. Discs 6.5 mm and 8.0 mm in diameter made of the same paper and dosed with the same amount of antibiotic will usually produce zones of inhibition of different diameters. But this difference will not necessarily be 1.5 mm which is what the FDA[4] assumes in applying their correction for a difference in the diameters of standard and test discs. The extent of the difference in zone diameter varies for different antibiotics. For instance mean differences of 0.98, 0.85 and 0.42 mm in zone diameters were obtained for 6.5 and 8.0 mm discs of Whatman 3MM paper dosed with 30 μg neomycin, 300 u polymyxin and 30 μg cephalothin respectively.

It would therefore seem that if we are aiming towards standardisation in that all discs of similar label claim should, irrespective of their source, perform similarly, then they must be assayed against a standard of both common paper and size.

UNIFORMITY:

The majority of the batches of Biolab and Difco discs that were tested had an acceptable degree of uniformity with pass rates of 87.5 percent and 94.1 percent respectively. However the other two manufacturers' pass rates were not acceptable. Only 40.0 percent of Alpha's batches tested and 56.3 percent of Mast's passed the specification.

Setting limits for within batch variation is fraught with difficulties. The FDA limits are set in terms of a maximum allowable range of zone of inhibition diameters. The difference between the smallest and largest zones produced by 90 percent of the discs sampled from a batch must not exceed 2.5 mm. The use of this type of criterion to judge uniformity has a serious disadvantage. A 2.5 mm spread in zone diameters can represent various ranges in potency depending on the dose response slope for the particular antibiotic. To illustrate this point the typical steep dose response slope of gentamicin can be compared with the shallower slope of methicillin (Figure 1). It is immediately obvious that a 2.5 mm difference in response (zone diameter) represents a much wider difference in dose (potency) for gentamicin than for methicillin. Consequently methicillin discs would have to be manufactured to much tighter limits, as far as content variability goes, than gentamicin discs to pass the FDA uniformity requirements.

One alternative form of judging the uniformity of a batch is to estimate the potencies of individual discs and express the maximum and minimum estimates as a percentage of the mean potency estimate. This method, limiting variation to 33 percent around the mean, was used in this survey. It seemed a fairer criterion than one based on the actual zone diameters because it is less dependent on the dose response slope of the assay system. However it has to be acknowledged that antibiotics with a steep dose response slope may be prejudiced against as any inaccuracy in reading the zone diameter transcribes to an appreciable discrepancy in the potency estimate.

Perhaps the ideal limits for uniformity should be individualised for each particular antibiotic in a particular assay system and based on the degree of uniformity that is achievable with the standard discs.

POTENCY AND UNIFORMITY:

Table 2 also shows the percentages of batches that passed the specifications for both potency and uniformity. It is clear that overall only Biolab and Difco discs are of sufficient quality.

The purpose of this survey was to decide if the quality of New Zealand manufactured discs had improved over the last five years and whether the New Zealand made discs compared favourably with some imported discs.

The disc now manufactured by Biolab is quite a different product to the disc they were producing and that we tested five years ago. They now make a 6.35 mm (¼") disc of a paper several times the thickness of the previously used paper which was cut into 9 mm discs. In the initial survey, in which the FDA limits were applied, 40 percent of the batches tested passed the potency limits and 60 percent the uniformity limits. Considering the tighter limits applied in this survey, the pass rate of 87.5 percent for both potency and uniformity indicates an improvement in the quality of Biolab discs. It is acknowledged that because different papers were used for the standard discs in the two surveys any comparisons of the potency pass rates are of dubious value.

The Alpha discs have changed little in appearance, still 8 mm in diameter and made of thin paper. After the first survey changes in production methods were implemented in an attempt to improve the qualaity, especially uniformity of their discs. However the results do not show that there has been any great improvement. Previous pass rates were 66.0 percent for potency and 23.8 percent for uniformity compared with 40.0 percent for both potency and uniformity obtained in this survey. In the first survey Whatman 3MM paper was used to make the standard discs which by coincidence is the same paper that Alpha were using for manufacture. Therefore for a true comparison the pass rate of 66.0 percent for potency obtained in the first survey should be compared with the 80.0 percent pass rate obtained in this survey when the discs were assayed against standard discs of the manufacturing paper.

The persistent feature of Alpha's discs has been the lack of uniformity. Alpha are currently changing their production and will be producing a smaller 6.5 mm disc made of Schleicher & Schuell paper 740-E and changes in methods will aim to improve the uniformity of these discs.

As expected the USA-manufactured discs, Difco, were of a high quality despite mishandling by the agent. This agent no longer holds the agency for Difco products. The UK-manufactured discs, Mast, did not reach the required standard.

Acknowledgements

We wish to thank the pharmaceutical companies who provided antibiotics for use as laboratory standards and Mr A. Carr for computer programming the analysis of results.

Published with the authority of the Director-General of Health, Department of Health, Wellington, New Zealand.

Table 1. Solvent, assay organism and media used for each assay

Antibiotic	Labelled content	Solvent	Assay organism	Medium
Ampicillin	10 μg	PO_4 buffer pH8.0*	*S. aureus* ATCC 13150	B.P. A pH6.6[5]
Cephalothin	30 μg	50% methanol	*K. pneumonia* ATCC 10031	B.P. A pH6.6/FDA E[4] †
Chloramphenicol	30 μg	50% methanol	*B. subtilis* ATCC 11774	B.P. A pH6.6
Clindamycin	2 μg	50% methanol	*B. subtilis* ATCC 11774	B.P. A pH7.8
Erythromycin	15 μg	methanol	*B. pumilis* ATCC 14884	B.P. A pH7.8
Gentamicin	10 μg	water	*B. subtilis* ATCC 11774	B.P A pH7.8
Kanamycin	30 μg	water	*B. pumilis* ATCC 14884	B.P. A pH7.8
Methicillin	5, 10 μg	water	*S. aureus* ATCC 13150	B.P. A pH6.6/FDA E
Neomycin	30 μg	water	*B. pumilis* ATCC 14884	B.P. A pH8.6
Novobiocin	5, 30 μg	water	*B. subtilis* ATCC 11774	B.P.A pH6.6
Penicillin	10 u	water	*B. aureus* ATCC 13150	B.P. A pH6.6/FDA E
Polymyxin	300 u	water	*B. bronchiseptica* ATCC 4617	FDA G/FDA F
Streptomycin	10, 25 μg	water	*B. subtilis* ATCC 11774	B.P. A pH8.1
Tetracycline	30 μg	methanol	*B. subtilis* ATCC 11774	B.P. A pH6.6
Tobramycin	10 μg	water	*B. subtilis* ATCC 11774	B.P. A pH8.1
Vancomycin	30 μg	water	*B. subtilis* ATCC 11774	B.P. A pH6.6

* Phosphate buffer pH8.0 used to dissolve powder, all subsequent dilutions in water.

† Different media used for base and overlay. Overlay medium described first.

Table 2. Percentage pass rates

Manufacturer	Number of batches tested	Percentage of batches that passed specification for: Potency	Uniformity	Potency and Uniformity
Alpha	15	40.0 (80.0)	40.0	6.7 (33.3)
Biolab	16	87.5	87.5	75.0
Difco	17	94.1	94.1	88.2
Mast	16	50.0	56.3	25

Figures in parentheses show the results when assayed against the standards made of the manufacturing paper.

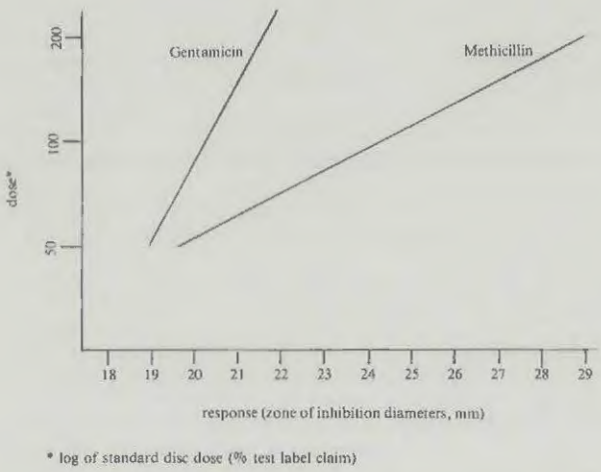

Figure 1: Dose/response slopes of standard Gentamicin and Methicillin discs.

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Actinidin—the Proteolytic Enzyme from Kiwifruit as an Aid to the Detection of Blood Group Antibodies

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Abstract
Trypsin, Papain, Bromelin and Ficin have been in use for a number of years as a proteolytic enzyme in Blood Group Serology. The proteolytic enzyme Actinidin extracted from the fruit of Actinidia (Kiwi fruit) is shown to have similar properties although a very short shelf life once activated.

Key Words
Proteolytic Enzymes, Actinidin, antibody detection.

Introduction
The Department of Scientific and Industrial Research (D.S.I.R.) Division of Horticulture and Processing have been determining the possibilities of commercial scale utilization of actinidin from kiwifruit. Small quantities of powdered enzyme were made available for investigation of its uses in blood group serology.

Method
Pure actinidin was obtained by salt precipitation of pulped actinidia (kiwi fruit) followed by dialysis against 10 mM phosphate buffer pH 6.5 containing 8 mM cysteine, 0.8 mM sodium metabisulphite prior to freeze drying. This actinidin was made up in concentrations of 0.5 percent to 2 percent and pH ranges of 5.59 to 7.38 and activated with either 1M cysteine hydrochloride, 1M sodium metabisulphite or 2 mM dithiothreitol at both 20°C and 37°C. These solutions were initially tested with an anti D known to work by other (papain, ficin) techniques but not active by saline 37°C techniques against a red cell panel containing D positive and D negative cells. A 1 percent solution of the dried actinidin powder in phosphate buffer of pH 6.98 activated with 1M sodium Metabisulphite was selected for further evaluation. After the addition of sodium metabisulphite a dense white precipitate was formed, this was removed by centrifugation and the clear supernatant used either immediately or was aliquoted and frozen or freeze dried. For use the 1 percent actinidin solution was incubated with an equal volume of serum and an equal volume of a 3 percent suspension in saline or Low Ionic Strength Saline (L.I.S.S.) of human red cells for 10 minutes at 37°C in a tube. After incubation and centrifugation at 3,000 rpm for 15 seconds the cell button was examined for agglutination using either a concave mirror or microscope.

Results
Initially the activity of actinidin solutions was only able to be detected for periods of up to one hour after the addition of any of the activating substances. After experimenting with concentrations of actinidin, pH variations and different activating substances a solution with a longer "shelf life" was obtained.

The 1 percent actinidin solution (pH 6.98) where used in parallel with a 1 percent cysteine activated papain solution (manufactured Taranaki Base Hospital) showed identical reactions on testing with a panel of human red cells and four examples of anti D, two of anti -c, two of anti E, one anti C and one each of anti Kell, anti M and anti Le^a. Two examples of anti Fy^a and one example of anti Jk^a showed no activity with either the actinidin or the papain reagents. Titrations of two anti D sera showed a two tube difference in end point in favour of papain treated cells (see Table 1). Activity of the 1 percent actinidin solution after 24 hours storage at room temperature was estimated by repeating the titres with anti D^1 where the papain titre was 256 (Score 93) and the actinidin titre 32 (Score 47).

Discussion
Proteolytic enzymes have been used to detect and identify antibodies to red cell antigens since Pickles[1] 1946 first reported that "a filtrate of a culture of vibrio cholera caused cells sensitised with an 'incomplete' anti Rh antibody to agglutinate". Since then trypsin,[2] ficin,[3] papain,[4] and bromelin[5] have been used as an aid to the detection and identification of some antibodies to red cell antigens. This limited investigation into the use of actinidin for such purposes has shown that 1 percent actinidin activated with 1M sodium metabisulphite at pH 6.98 showed similar properties to 1 percent cysteine activated papain when used to remove sialic acid from the red cell surface allowing agglutination to take place with appropriate antibody specificities. However, the lack of stability of activated actinidin solutions remains a problem as is shown by the reduced titres and titre score with the anti D both with fresh reagents and 24 hour stored reagent. Until this problem is overcome actinidin will remain a reagent with potential rather than a serious alternative to the existing blood group enzyme reagents available.

Table 1

	Papain		Actinidin	
	Titre	Score	Titre	Score
anti D^1	256.	97.	64.	71.
anti D^2	8.	26.	2.	13.
anti D^1 (24 hour storage)	256.	93.	32.	47.

Acknowledgements
The authors wish to thank Dr D. J. W. Burns, Department of Scientific and Industrial Research (Division of Horticulture and Processing) Auckland for his advice and for supply of the Actinidin powder.

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A Case of Hairy Cell Leukaemia

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Abstract

This paper describes a case of Hairy Cell Leukaemia detected during routine preoperative screening and details the various cytochemical and immunological tests carried out to confirm the diagnosis.

Introduction

Hairy Cell Leukaemia is a neoplastic disorder of the lymphoreticular cells characterised by the presence of the pathognomic hairy cell, a distinctive mononuclear cell that circulates in varying numbers and infiltrates a variety of organs including the bone marrow and spleen.

Hairy cells derive their name from their fine irregular cytoplasmic projections which are often indistinct being more conspicuous in cytospin preparations (see Figure 1) or by electron microscopy.

The incidence of the disease has been given as approximately 1 percent of all identifiable lymphomas or 2 percent of all leukaemias;[1] although two other cases of hairy cell leukaemia have been confirmed in our laboratory this year.

Discussion

CLINICAL ASPECTS

The overall mean age at presentation is 51 years with a male : female ratio of 4:1. Up to 75 percent of patients present with non-specific symptoms such as weakness, weight loss and dyspnoea,[1] while a smaller percentage may present with infection, haemorrhagic tendencies or may even be found incidentally. Splenomegaly is the most common physical finding with hepatomegaly and lymphadenopathy being less frequently encountered.

HAEMATOLOGICAL ASPECTS

Characteristic haematological findings in untreated Hairy Cell Leukaemia are those of bone marrow failure, i.e., anaemia, neutropenia and thrombocytopenia. The most variable parameter is the total leucocyte count with the percentage of circulating hairy cells increasing with the total leucocyte count. The ESR is variable with marked elevation commonly associated with infection.

Nearly all patients have a neutropenia (i.e., less than 2.5 x 10^9/l), a monocytopenia may also be seen in many cases. A moderate normocytic normochromic anaemia with an increased MCV is common, although this macrocytosis is not normally accompanied by reduced serum B12 or folate levels or marrow megaloblastosis.

Thrombocytopenia is present in 85 percent of patients[1] with platelets showing qualitative as well as quantitative abnormalities.

The bone marrow is nearly always involved but usually proves inaspirable. Trephine biopsies are often called for to demonstrate hairy cell infiltration which may be from 50-90 percent. The cells are often less "hairy" than in peripheral blood. Generally granulocyte and monocyte precursors are reduced, while megakaryocytes and cells of the erythroid series appear normal.

OTHER INVESTIGATIONS

A slightly elevated serum alkaline phosphatase, decreased albumin and increased alpha-2 globulin may be found.

IMMUNOLOGICAL ASPECTS

Despite intensive investigations, the origins of hairy cells are still elusive. Hairy cells share marker characteristics with monocytes, B lymphocytes, T lymphocytes and third population or null cells as well as having several distinctive features. This suggests that they may be derived from an undefined counterpart.

Prognosis

Hairy Cell Leukaemia is usually a chronic disorder but may run an acute or subacute course. The median survival is fifty months although the range is wide and survival of 27 years has been documented.[1] There is a significantly longer overall survival in patients who have been splenectomised despite the accompanying risk of post-operative infection. After splenectomy marked fluctuations in the course of the disease accompanied by the disappearance of circulating hairy cells are often observed.

The presence of a leucocytosis at presentation may possibly be of worse prognosis but the degree of marrow impairment is by far the most important prognostic feature. If patients survive the first two years of the illness survival is often relatively lengthy with infection being the major cause of death.

Case History

In March of this year, the propositus, a sixty-eight year old male Caucasian, was admitted for the removal of a lipoma from the left lumbar region, a purely cosmetic procedure. The fatty tumour had been present for twenty years and was uncomfortable while driving. A routine preoperative screen showed a mild macrocytic anaemia. The differential showed 74 percent hairy cells (See Table 1).

Consequently the operation was postponed while further investigations were carried out to confirm the initial diagnosis of hairy cell leukaemia.

On re-examination the patient was found to have an enlarged spleen, easily palpable 8 cm below the costal margin. There was no lymphadenopathy or any evidence of current infection. The liver was enlarged 6 cm below the costal margin.

A bone marrow aspiration and trephine biopsy were performed. The myelogram revealed 16 percent of all nucleated cells to be small lymphocytes and 54.5 percent showed atypical nuclei, ground glass cytoplasm and hazy cytoplasmic borders, consistent with hairy cells.

In the trephine biopsy, the bony trabeculae were abnormal with prominent osteoclastic activity and new bone formation. The marrow was hypocellular with a diffuse open infiltration of atypical mononuclear cells.

The reticulin was mildly increased. In conclusions the marrow showed appearances of: Hairy cell leukaemia, and Paget's disease of the bone.

TREATMENT

The patient was splenectomised at the beginning of the fourth week following this incidental finding. There was a subsequent drop in the total leucocyte count and a marked decrease in the number of circulating hairy cells (see Table 1). The platelet count increased and other typical post-splenectomy findings were noted.

Five months after presentation the white cell count and hairy cell percentages peaked at 18.6 x 10^9/l and 43 percent respectively.

The patient's progress is being followed with interest and is being monitored on an outpatient basis.

Methods and Results

Routine haematological studies were performed with the Coulter Model S. The following cytochemical stains were performed using standard methods. Butyrate esterase,[2] acid phosphatase (tartrate resistant),[3] neutrophil alkaline phosphatase.[4]

Further tests include: Sheep Cell Rosettes,[5] Surface Immunoglobulin,[6] Mouse Erythrocyte Rosettes,[7] and Cytoplasmic Immunoglobulin.[8]

The Neutrophil Alkaline Phosphatase score was markedly raised giving a score of 260. Serum alkaline phosphatase was also slightly raised. Protein electrophoresis revealed a slightly increased alpha-2 globulin.

The abnormal cells were identified as hairy cells by:

(a) Acid phosphatase tartrate resistant cytochemistry. The acid phosphatase stain was strongly positive, after exposure to tartrate the reaction was only weakly positive. This is unusual as it is generally accepted that the tartrate resistant acid phosphatase (TRAP) activity (which characterises isoenzyme five) is stronger in hairy cells than in any other cell type.

(b) Non-specific esterase, using butyrate esterase and alpha napthyl acetate as substrates.

The patient's hairy cells showed a distinctive pattern of butyrate esterase staining with scattered fine granules and coarser granules localised in a crescentic pattern (see Figure 2). This reaction is now considered to be of greater diagnostic significance than the TRAP reaction which is being found to be negative in an increasing number of cases.[9]

(c) Surface Immunoglobulin, Cytoplasmic Immunoglobulin and mouse red cell rosetting.

Hairy cells, like B lymphocytes produce surface immunoglobulin and show mouse erythrocyte rosetting. Sixty-four percent of the total cell layer tested showed production of Kappa light chains, and 40 percent showed rosetting with mouse red cells. A large proportion of cells showed the production of cytoplasmic immunoglobulin which is not found in normal T or B cells. This has been reported in only 5 percent of cases of Hairy Cell Leukaemia.[1]

(d) Scanning and Transmission Electron Microscopy.

Transmission electron microscopy showed the presence of ribosomal lamellar complexes. Although these complexes may occasionally be seen in a variety of other cell types they are more common in Hairy Cell Leukaemia than in any other haematological malignancy and are therefore of diagnostic significance. These complexes are composed of ribosome granules enclosed in an elaborate coiled network of fibrils. Their function and origin are not known (see Figure 3). Scanning electron microscopy showed a combination of characteristic ruffles, ridge-like projections and narrow finger-like microvilli (see Figure 4).

Conclusion

Hairy Cell Leukaemia is a recognised specific disease entity with distinct clinical and haematological aspects that can almost always be diagnosed with a relatively few simple investigations. Diagnosis centres on the recognition of the pathognomic Hairy Cell which is never completely absent from the peripheral blood at presentation. Accurate diagnosis has important therapeutic implications for if erythropoiesis is not impaired splenectomy rather than chemotherapy is usually the treatment of choice.

Fig. 1. A cytospin preparation at 1000 x magnification showing the fine irregular cytoplasmic projections.

The mononuclear hairy cells are generally larger than most lymphocytes with a diameter of 15-30 μm. They have a round to oval, sometimes folded nucleus with fine chromatin and one or two small nucleoli.

The cytoplasm is slate blue, usually homogeneous or finely mottled and occasionally vacuolated. There is a single normal lymphocyte for comparison.

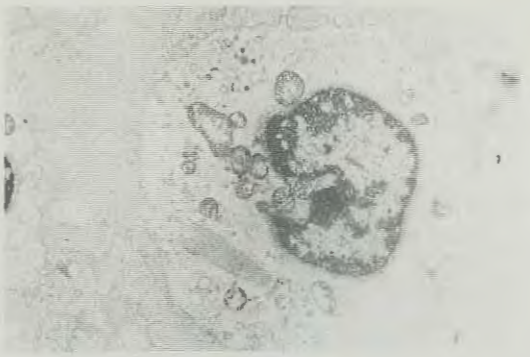

Fig. 2. Hairy cells showing crescentic pattern of butyrate reaction.

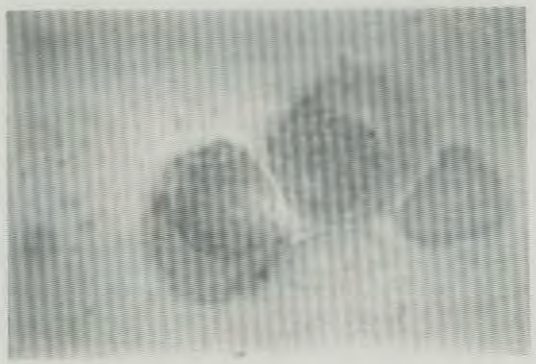

Fig. 3. Transmission electron microscopy showing presence of a single ribosomal lamellar complex.

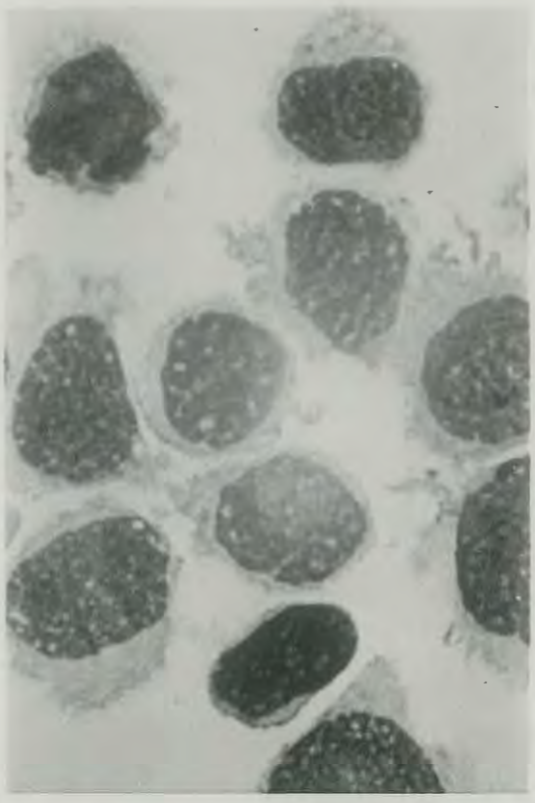

Fig. 4. Scanning electron microscopy showing a single Hairy Cell.

Table 1: Shows the patient's peripheral blood changes from presentation to after splenectomy.

	Red Cell Count x 10⁹/l	White Cell Count x 10⁹/l	Haemoglobin g/l	Platelets x 10⁹/l
Day 1	4.0	11.4	119	153
Day 3	3.8	11.8	121	182
Day 4	3.9	14.9	130	
Day 6	4.0	15.6	129	142
Day 20	3.5	11.4	119	89
Day 21	3.7	12.6	125	87
Splenectomy				
Day 23	3.2	11.5	108	88
Day 27	3.3	6.6	111	285
Day 51	3.4	6.7	113	270

DIFFERENTIAL

	Hairy Cells %	Neutrophils %	Lymphocytes %	Monocytes %
Day 1	74	3	21	2
Day 3	67	6	26	1
Day 4	62	10	27	1
Day 6	52	12	36	
Day 20	70	6	24	
Day 21	63	4	32	1
Splenectomy				
Day 23	51	23	25	1
Day 27	21	20	52	3
Day 51	11	21	62	2

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Acknowledgement

We acknowledge the help of Zygmunt Poczwa of the Electron Microscopy Section, Dunedin Hospital, without whose help this paper would not be possible.

Medical Laboratory Technologists and Continuing Education

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Abstract

On-going or continuing education provides an opportunity to update knowledge and skills that have been acquired over the years. It also brings about changes in the attitudes for more effective performance. New techniques and rapid expansion of methodologies keep pouring into the laboratories of today. The knowledge that was acquired yesterday becomes obsolete and as such it is essential for Medical Technologists to be better equipped to meet the new challenges that are forthcoming. This new role has necessitated a need for continuing educational efforts and currently, numerous continuing educational programmes are available ranging from university education to seminar workshops. Existing continuing educational programmes are reviewed. Apart from the full time university curriculum, the most widely utilised form of continuing education appears to be short term educational formats involving small groups of participants.

Key Words

Continuing education. Medical Technologists. Learning. Postbasic or postgraduate studies. Laboratory.

Introduction

Continuing education is a subject that has been talked about at all times and by many people. Over a century ago Florence Nightingale observed that nursing is a progressive art in which to stand still is to go back. Sir William Osler, one of medicine's great leaders, advised physicians to discontinue practice every fifth year and return to hospitals and laboratories for renovation, rehabilitation, rejuvenation, reintegration and resuscitation.[1] This advice should also be applicable to Medical Technologists and they should be encouraged to participate in short courses in his or her speciality at least once in three years.

On-going or continuing education not only provides an opportunity to update knowledge and skills that have been acquired over the years but to learn new ones. It also brings changes in the attitude for effective performance. Medical Technologists of today are confronted with many new techniques and rapid expansion of methodologies.

The knowledge that was acquired yesterday becomes obsolete. For example, the estimation of blood haemoglobin by Tallqvist's scale using blotting paper and a series of colours representing haemoglobin concentration has been superseded by press button automation. Due to changes resulting from scientific advances, new ideas and information keep pouring into the laboratories of today. Participation in continuing education will provide an opportunity to improve oneself so that he or she is well-equipped to meet the new challenges ahead and keep abreast of changes.

Routes of Qualification of Becoming a Medical Laboratory Technologist

When the subject of continuing education for Medical Technologists is dealt with, it is important that the preparatory education for Medical Technology be also considered. It is evident

that there are a number of qualifying routes to becoming a medical technologist. Many developed and developing countries like Australia, England, Japan, the United States of America, the Philippines, Taiwan and Thailand offer full-time university courses leading to Bachelor of Science degree in Medical Laboratory Sciences. In other countries, a two or three year full-time diploma or certificate course is conducted in special schools solely utilised for training of Medical Technologists similar to that of the School of Medical and Health Laboratory Technology in West Malaysia.[2] Medical Technology is also taught in other countries as an in-service course or on-the-job training with examination and accreditation. These varied routes of qualification have their merits and demerits when continuing education is organised.

Different Forms of Continuing Education

Since early days, various forms of continuing education programmes have been conducted for Medical Technologists. Listed below are some of the different forms of continuing education available currently:

(1) Postgraduate studies in universities
(2) Short term, small group refresher courses
(3) Scientific meetings and seminar workshops
(4) Self-directed studies.

Postgraduate Studies in Universities

One widely accepted form of continuing education today for Medical Technologists who initially entered the profession with basic university degrees is the postgraduate studies in universities and institutes of higher learning. All institutes which offer basic degree courses in Medical Laboratory Sciences also prove facilities for postgraduate studies. These institutes offer a definite predetermined curriculum in many disciplines of clinical and laboratory medicine.

Short Term—Small Group Refresher Courses

Another means of continuing education for Medical Laboratory Technologists is the short-term small group refresher courses covering specific areas of Medical Laboratory Sciences. As the subheading suggests these courses are of short duration and comprise a small group of participants. This would lead to a close rapport being established between the instructor and participants.

Being of short duration and covering specific areas, many would like to participate. One of the major problems in organising short term courses is either an overwhelming response due to low registration fee and popular topics or poor response because of expensive registration fee. In the former instance, short listing candidates is a difficult task, while in the latter instance courses have to be called off or open free to any participants and expenses incurred have to be borne by the organisers.

Scientific Meetings and Seminar Workshops

Year in and year out scientific meetings, workshops and seminars are organised in various forms by different organisers. The very purpose of attending a congress similar to that of the South Pacific Congress is in itself a continuing education. More often than not this fact is forgotten. Trade exhibitions, workshops and scientific programmes provide ample opportunity to learn many new things in a short period of time. Scientific meetings provide a platform where research results and new discoveries are thrown for discussion and comments. These meetings usually end up in lively forums where many ideas and concepts are debated and finally carried back to the place of origin by participants. It involves years of planning and dedication of many people to organise a successful congress. While the success or failure of a congress may depend largely on the number of participants, the registration fee and other costs are also the governing factors.

Self-directed Studies

Continuing education can be pursued by way of engaging in self-directed stuides. Besides self-motivation, books and journals are useful tools to indulge in self-directed studies. Forming the habit of reading in the library is important in order to perform self-directed studies. This could best be done by including a topic on the usefulness of library and library facilities in the syllabus of Medical Technology curriculum. Trainee Medical Technologists, be it at undergraduate level or in the laboratories doing in-service courses, must be taught the usefulness of the library for career development. Most libraries of today provide almost all new books and the latest issues of journals in the field of Medical Technology. As a result, unlike other forms of continuing education, library facilities do not require heavy budgetary support of pre-requisite qualification for on-going education.

Comments

Among the various forms of continuing education considered, postgraduate studies are most preferred. Other forms of continuing education have their effect in improving the efficiency of Medical Technologists to provide better service to the patients and the health care delivery system as a whole. However, it does not provide an opportunity to elevate oneself in career advancement. Continuing education depends very much on the type of preparatory education provided and as long as varied approaches to preparatory education are adhered to, it will continue to pose problems in organising postbasic or postgraduate studies. Medical Technologists of today besides being encouraged to participate in continuing education are faced with many threats posed by automation. In the first place they are on the verge of losing their jobs to machines. In many areas of clinical medicine, laboratory workers only have to press buttons of automatic machines and the results are threre. This has resulted in frustration among Medical Technologists because their specialised skills and creativity are no longer required and the profession becomes unchallenging to many. Since Medical Technologists are closely linked with machines, there is a need to develop special skills to maintain and repair the machines, failing which many will lose their jobs to engineers.

Conclusion

Accepting university or university equivalent degrees as routes of qualification to becoming MLTs as recommended by the Standing Representative Committee for Medical Technologists in European Economic Community is a probable solution to the problems in organising postbasic or graduate studies.[3] It has been also emphasized that the other qualifying routes to become a medical laboratory technologist be improved. There is an urgency in reviewing the present Medical Technology curriculum so that more topics and time be allocated to the teaching of electronics, instrumentation and computer science. Incentives in the various forms of paid educational leave, accumulation of annual leave for continuing education, and conference leave to attend scientific meetings and seminars should be provided to medical technologists. The need for continuing education for Medical Technologists is well established and accepted. As such every endeavour should be made to make continuing education attractive, stimulating and all the more rewarding.

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Acknowledgement

The author wishes to thank Dr A. J. Tilagachandran, Department of Social and Preventive Medicine, Faculty of Medicine and Associate Professor T. Marimuthu, Faculty of Education, University of Malaya, for reviewing the paper.

This paper was presented at the 1st South Pacific Congress, Christchurch, New Zealand, 11th-13th August 1982.

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WN9

Technical Communication

An Economical Quick Thaw Cryoprecipitate Shaking Bath

Les Milligan, Heather Kerr, and Len Foley
Immunohaematology Department,
Public Hospital, Dunedin,
New Zealand.

A Baird and Tatlock continuous shaker and a standard laboratory waterbath were adapted in order to produce an economical controlled thaw-shaking water bath for use in cryoprecipitate production.[1]

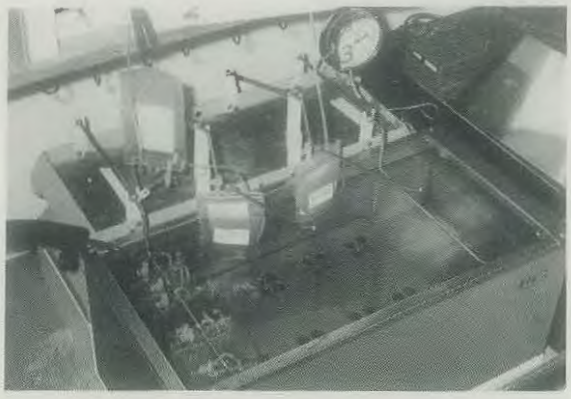
The continuous shaker was originally belt driven from a motor with a 1:1 ratio, but this was altered to a chain drive motor with a ratio of 2.3:1. A rheostat provides variable speed control and is set to allow 130 cycles per minute.

The thawing bath is a stainless steel BTL water bath which is filled with tap water. The stainless steel used for the tank is highly resistant to corrosion, the seams are welded rather than soldered. Splashing is virtually eliminated by using a deep tank with the water level eight centimetres below the top of the bath. The packs are almost completely immersed, with the ports kept above the water level at all times during thawing. The stainless steel rods, which support the packs in the thawing bath, are held in place by hinged flat clamps which are attached to the shaking deck. The support rods are raised for loading and unloading, and are lowered to immerse the packs during thawing.

A Grants Instruments freezing unit, model CS15, is used to maintain the temperature between 4-8 °C during thawing. The temperature is monitored by an electronic temperature gauge. The coil of the cooling unit fits under the circulation tray of the bath and therefore does not obstruct the working space. Water is cooled continuously as it passes under the tray and the desired temperature is maintained by the control system. The water is kept in constant agitation by action of the moving packs.

The waterbath and shaker are mounted on a solid wooden stand to prevent vibration. Operator safety is assured by using a totally enclosed shaking-drive mechanism.

Twenty-four units of Fresh Frozen Plasma may be thawed at once and plasma thawing is complete within two hours. Provision is made, if required, to keep the attached red cells at a constant 4 °C.



Preliminary work on quick thaw cryoprecipitate has shown an average yield of 110 units per bag. Subsequent reviewing indicates that this yield can be improved.

This module has been frequently used and can be manufactured from readily available laboratory equipment.

Alterations to the equipment were carried out by the Medical Equipment Workshops of the Dunedin Hospital.

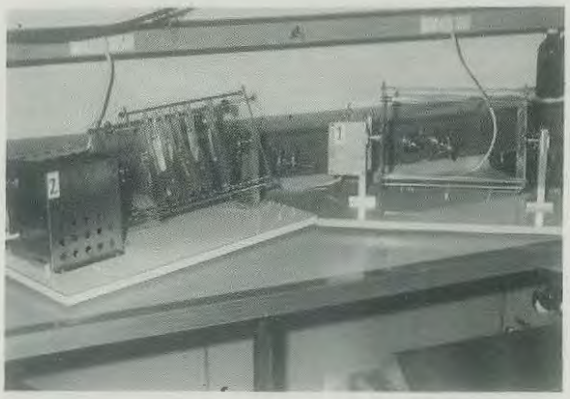
1. Sibinga, S. et al, *Lancet*, August 1981.

A Simple Economical Platelet Rotator

Les Milligan, Alan Knight, Heather Kerr, and Ralph Harvey

Immunohaematology Department,
Public Hospital, Dunedin,
New Zealand.

A rotator may be used for the convenient storage of single donor and pooled platelet concentrates. If the temperature is maintained between 20-24 °C, the rotator assures constant mixing at uniform pH. This equipment is often expensive and beyond the budget of smaller laboratories. A simple, inexpensive yet effective model was designed and built locally.[1]



The small, second hand electrical motor, used to rotate the module, has a 14 watt output. The wormwheel and gear drive reduced the 1428 r.p.m. to 30 r.p.m. A cooling fan for the unit runs from the same motor. The motor housing is made of perforated stainless steel to aid the cooling and the motor and fan attachment is conveniently arranged for operator and product safety.

The plasma unit carrier consists of two perspex sheets held in place by aluminium uprights (175 mm x 38 mm x 12 mm) and is held apart by stainless steel rods 330 mm in length. The main drive spindle is supported by the aluminium uprights and is in turn supported by bearings (28 mm diameter x 10 mm width) housed in the uprights. The packs are held by retractable stainless steel rods threaded through the perspex end plates. The rods are held in place by metal spring clips. The rotator holds up to twelve bags of platelet rich plasma and ensures constant mixing of platelets at a uniform pH with gentle rotation and aeration.

This unit is mounted on a plastic laminate covered board. The materials for this unit are both non-corroding and easily cleaned.

The manufacture of this unit is a relatively straightforward project for a Precision Technician and provides a cost effective unit.

1. AABB, Technical Manual, 8th Edition, 1981.

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MEDICAL LABORATORY SCIENCES VOL. 39 NO. 2.

The monoclonals are coming.

Monoclonal anti-A and anti-B: development as cost-effective reagents

Mouse monoclonal anti-A and anti-B as routine blood grouping reagents: an evaluation

β_2 microglobulin and the analytical characterization of proteinuria

The ascorbate cyanide test and the detection of females heterozygous for glucose-6-phosphate dehydrogenase deficiency

Extraction and recovery of IgG antibodies using *Staphylococcus aureus*

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Action of formaldehyde upon plate cultures of various bacterial species.

How publishing medical microbiologists acquire information.

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The production of anti-P_1 in rabbits immunised with pigeon ovomucoid.

Collection of epoxy resin sections for light microscopy using a large water-filled trough

A selective and differential medium for group B streptococci.

Haemophilus influenzae and *H. parainfluenzae*: the influence of media and CO_2 on differentiation using X, V and XV discs.

A rapid method for detection of *Trichomonas vaginalis*.

LABORATORY MEDICINE VOL. 13 NO. 5.

(1) Erythroblastosis fetalis—A review.
(2) Glycol Methacrylate Embedding Technique.
(3) Platelet Satellitism.
(4) Storage of Blood Donor Library Samples.

AMERICAN JOURNAL OF MEDICAL TECHNOLOGY VOL. 48. NO. 6.

(1) Haematologic Morphology; including historical review of toxic and reactive changes in granulocytes and monocytes; artefactual alteration and a review of Romanovsky stain.
(2) Use of Gram Stain to Predict Vitreous Fluid Infection.
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(2) A Three-Part Performance Appraisal System.
(3) The Bg antigen in Amish Pedigrees.
(4) A Modified Fluorochromatic Cytotoxicity Test for the Study of Leukaemia Associated Antigen and Antibodies.
(5) A Microleucoagglutination Assay for the Study of Leukaemic Myeloblast Antigens Using Monkey Antiserum.
(6) Platelet Behaviour During Cardiopulmonary bypass: A Comparison of Four Bubble Oxygenators.

BOOK REVIEWS

Laboratory Investigation of Fetal Diseases. Edited by A. J. Barson, Published by John Wright and Sons, Ltd, Bristol 1981 (504 pages). Obtained from A.N.Z. Book Co., P.O. Box 33406, Auckland 9.

This book was written for the clinical perinatologist, and contains 21 chapters contributed by 35 authors who are an international group of experts. Each chapter forms a concise summary of recent developments and current practices in one "laboratory" area including physiology, radiology, clinical pathology, haematology, microbiology and genetics. The degree of detail is sufficient for the clinician, but not for a laboratory worker actually involved in the techniques of investigation.

The first section of the book is concerned with monitoring for fetal growth and distress, and covers fetal radiology, oxygen tension, heart rate, breathing and electroencephalograms, as well as plasma and amniotic fluid hormone assays.

The second section concerned with antenatal diagnosis of disease has chapters on cytogenetics, alpha-feto-protein screening, metabolic disease, ultrasound and radiology, prediction of haemolytic disease and respiratory distress syndrome, fetoscopy and diagnosis of haemoglobinopathies.

The third section on the postnatal diagnosis of disease considers intra-uterine infections, population screening for inherited metabolic disorders, their clinical management, and three thoughtful chapters about post mortem investigations.

The chapters are concise, well written, and well referenced. The illustrations are of good quality. Because of the up-to-date nature of the material presented, the book will necessarily become outdated, but as stated in the foreword by Prof. J. A. Davis, it is a "summing up for the next year or two of the usable knowledge that has been accumulated".

The book is a successful attempt to draw together information from a large number of fields and presented in a reasonably digestible form for those of us not actively engaged in laboratory work or research. Laboratory workers may well enjoy the chance to catch up in fields other than their own.

I certainly recommend this book to people working in the perinatal field.

J. E. Clarkson,
Paediatrician.

Disinfection, Sterilization and Preservation. Edited by Seymour S. Block. Published by Lea & Febiger. Obtained from A.N.Z. Book Co., P.O. Box 33406, Auckland 9.

This textbook sets out to cover a very wide ranging topic as its title indicates. The Editor, Dr Seymour Block and the contributors achieve this aim in a book which seems to cover all aspects of antimicrobial agents and processes. To do so the book is divided into seven parts covering—Methods of Testing, Antiseptics and Disinfectants, Chemical and Physical Sterilization, Medical and Health Related Applications, Antimicrobial Preservatives and Protectants, Mode of Action and a final section covering "Additional Topics" which includes control facilities, definitions and an historical review.

The 55 contributors are all experts in their field and hence this test brings together consider expertise and a wealth of information in its 1,000 plus pages. The print is easy to read and there are a large number of tables, figures, pictures and diagrams which help in illustrating the various points being made. Each chapter concludes with a very extensive bibliography. Because the authors are covering the whole area of antimicrobials for the whole range of biological sciences there are areas of the book which are not directly relevant to medical applications but this does broaden the horizon of knowledge and reminds us that there are other areas using similar methods and trying to grapple with problems similar to ours.

Most of the methods of testing given in detail are those approved by the F.D.A. and therefore may not be those we use, especially as far as "in-use" testing of disinfectants. The chapter dealing with specific disinfectants gives considerable detail of their biochemical structure and uses generic taxonomy and does not mention trade names. This can be a disadvantage when looking for information on a specific agent.

The chapters dealing with Chemical and Physical Sterilization are full of very worthwhile information and would be useful as an adjunct to the usually read texts on these subjects. The chapter on Spacecraft Sterilization may not be very relevant in our country but is interesting reading.

Two other chapters are worthy of mention. That on the "Treatment and sanitary disposal of infectious hospital wastes" gives details on sterilization and incineration procedures and reinforces the necessity for rendering hospital waste safe before disposal to safeguard the community and environment. That covering the "Hazard of infectious agents in microbiological

laboratories" reminds us of the potential hazards in the laboratory with hepatitis, tuberculosis and brucellosis heading the list of causative organisms, in 1972, of 3,497 reported laboratory infections of which 4.6 percent were fatal.

Although this book would not be one which every Microbiologist would have in his own personal library, it is certainly recommended as a reference text which would be a very useful addition to the laboratory library, especially in teaching laboratories and those in which the staff have a responsibility for advising on disinfection and sterilization procedures within the hospital or institution.

John Elliot

Diagnostic Microbiology. Sydney M. Finegold: William J. Martin. 6th Edition, 1982. Mosby. Obtained from Peryer Educational Books (S1), P.O. Box 6034, Christchurch 4.

The appearance of the new sixth edition of Bailey and Scott's Diagnostic Microbilogy this year will be welcomed by the many medical laboratory technologists who have over the years developed a high regard for this textbook.

The new edition however marks the beginning of a new era with a change of authors. Sydney Finegold and William Martin state that they readily accepted the invitation by Elvyn Scott to become the new authors of a textbook they had long regarded as a classic in its field.

Sydney Finegold is Chief of the Infectious Disease Section, V.A. Wadsworth Medical Centre and Professor of Medicine, UCLA School of Medicine.

William J. Martin is the Director, Microbiology Laboratory, Tufts—New England Medical Centre, Professor of Pathology, Tufts University School of Medicine, and formerly Professor of Pathology and of Microbiology and Immunology, UCLA School of Medicine.

The book which now has 705 pages, and includes 193 illustrations and 39 colour plates, has been re-written, partly re-organised, and completely updated. Examples of new material which has been added, or topics which have been significantly updated and extended in this volume include, new methods for detecting bacteriuria, the role of chlamydia, new classification of Enterobacteriaceae, non-fermentative gram-negative bacilli, newly described mycobacteria, rapid processing of anaerobes, Legionnaires' disease, antimicrobial induced colitis, toxic shock syndrome, Kawasaki disease, and nutritionally variant streptococci.

The book is divided into eight parts.

Part I gives a brief superficial description of laboratory methods including the sterilisation of media, optical methods in specimen examination, staining procedures, and methods of obtaining pure culture.

Part II is a useful section dealing with the philosphy and general approach to clinical specimens, and their collection and transport.

Part III deals with the normal flora, potential pathogens and the handling of samples from the more important anatomical sites. This is an excellent section particularly the chapter relating to blood culture which includes some interesting comments on the use of the Antimicrobial Removal Device (ARD).

Part IV is devoted to the identification of pathogenic micro-organisms and includes a broad spectrum of common and more unusual pathogens, bacterial, viral and fungal. The final chapter in this section dealing with the lahoratory diagnosis of parasitic infections was written by Lynne Garcia who will be remembered for her excellent contribution as a guest speaker at the South Pacific Congress. This is a very good section, concise, relevant, up-to-date and of particular value to the practising microbiologist.

Part V is concerned with antibiotic susceptibility testing and the assay of antimicrobial agents. It contains a useful table giving the "breakpoints" of the commonly used antimicrobial agents and an account of dilution and agar diffusion tests, and the susceptibility testing of anaerobes. These topics are adequately covered but the assay of antimicrobial agents receives very superficial treatment.

Part VI outlines serologic methods in diagnosis and the identification of micro-organisms. Techniques described include coagglutination and ELISA.

Part VII consists of the two chapters. The first gives a brief but useful outline of automation and rapid methods such as gas chromotography, autobac, dynateck MIC-2000, pepliscan system and the automicrobic system.

The second chapter deals with quality control and safety and includes an excellent table listing the criterion for the rejection of specimens and the action to take, an area which most textbooks neglect.

Part VIII details the formulas and preparation of culture media.

The new edition of Diagnostic Microbiology is a quality hardbacked textbook with many excellent illustrations, colour plates and tables. Value for money this book would be hard to beat. It is not uncommon for books of this type to be dated by the time they are published. The new authors have ensured that this is not the case with Diagnostic Microbiology. This textbook would be of value not only as a benchbook but also to students through to a Specialist Certificate level.

Laboratory Medical Mycology. By Yousef Al-Doory. Published by Lea & Febiger. 410 pages. Obtained from A.N.Z. Book Co., P.O. Box 33406, Auckland 9.

This book is aimed to be useful to clinical and laboratory staff alike.

The beginning of the book covers well the basic general rules of such aspects as Safety, Quality Control and Microscopy with appropriate emphasis on their application to mycology.

The chapter on specimen collection begins well with the important fundamental requirements for collection of good mycological specimens followed by two tables indicating (1) specimens and, (2) recommended amounts of specimens from specific sites. However the remaining 20 odd pages of the chapter covering each site in detail is, I feel, over full. For example I do not consider it necessary to have included detailed instructions as for the technique of venepuncture in a book such as this is entitled.

Specimen examinations and processing is also thoroughly covered. Probably a little too thoroughly for the laboratory technologist but necessarily fuller for the clinician wishing to have a working knowledge of laboratory techniques. There is excellent coverage of the choice of direct microscopy reagents, media and stains—each of which has a conclusion stating its use or usefulness.

There is short concise coverage of those diseases previously thought to be fungal. Aerobic and anaerobic actinomycetes are covered in separate chapters. The author now beginning to cover more specific topics presents the material in a superior fashion with more use made of subheadings and tables of information.

Mycoses is covered in two separate sections:

1. Generally with clinical pictures encountered, principal causative agents expected and specimens required.
2. Laboratory identification characteristics of separate species. There is no scheme or key to follow to aid identification for dermatophytes although there is one for yeasts.

   The yeast sections also contain tables of characteristic biochemical reactions and identification procedures prior to specific characteristics of individual species.

Opportunistic fungi are covered more fully than many other mycological textbooks.

There is a small amount of pictorial material in the form of high quality black and white photographs.

A. Paterson.

NEW PRODUCTS AND SERVICES

AUTOMATED BECKMAN CLINICAL ANALYZER OFFERS FLAMELESS SODIUM AND POTASSIUM TESTING

Beckman Instruments, Inc. combines total automation with state-of-the-art Ion Selective Electrode (ISE) measurement methodology in the Electrolyte 2 Analyzer. The compact instrument also features microsampling, high throughput and self-diagnostics.

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For more information, contact Beckman Instruments New Zealand, P.O. Box 9057, Newmarket, Auckland, New Zealand. Telephone 64-9-836-9987.

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The ICS data processor is the operator's primary interface with the Auto ICS as all operational activities are automatic. A multiple-function 65-pad keyboard, two alphanumeric displays, a high-speed printer and versatile cassette programming make the system setup and programming quick and simple.

Available reagent kits for the Auto ICS are C-Reactive Protein, Properdin Factor B (C3-Pro-Activator), Ceruloplasmin, Alpha$_1$-Acid Glycoprotein (Orosomucoid), Alpha$_2$-Macroglobulin, IgM, IgG, IgA, C3 Complement, C4 Complement, Albumin, Transferrin and Haptoglobin. Rheumatoid Factor and drug assays for Phenytoin, Phenobarbitol, Theophyline, Gentamicin and Tobramycin.

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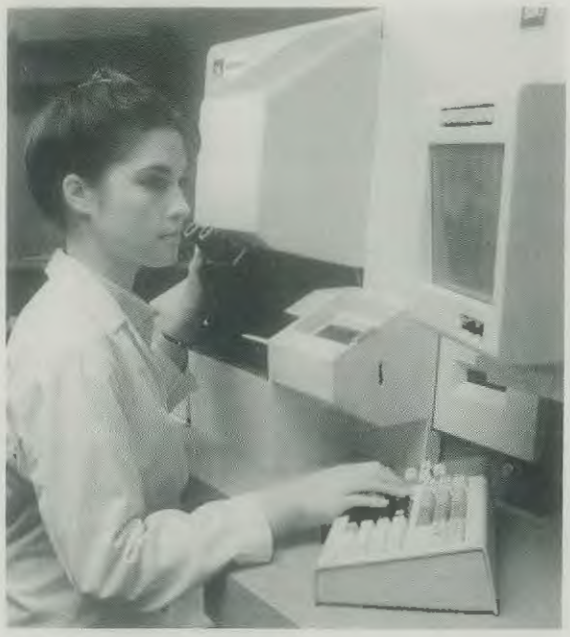
A new generation of Hematrak blood cell counting systems now available from CARL ZEISS is claimed to bring fully automated differntial counting facilities within the normal laboratory budget.

Four advanced systems are being introduced. The simplest type, model 450, performs normal and abnormal white cell differential, red cell morphology and numerical platelet estimates. It can process batches of up to 10 slides in an unattended mode at a rate of 45/h. At the top of the range, model 590 also produces Price-Jones curves, and lymphoctye profiles; it can automatically process a 100-slide load in one hour; and it features a bar-code reader for positive patient indentification.



The new models employ the proven high resolution three-colour pattern recognition technique, on which the success of the Hematrak range has been built. Their usefulness is further enhanced by the software controlled ReCap and ReCell modes which respectively enable data on specific abnormal cells to be identified and stored in a built-in memory file, and a patient-flagging alert facility with operator selectable threshold. All models also feature floppy-disk automatic software loading for easy reprogramming.

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For more information contact: Carl Zeiss Pty. Ltd.. Wellington: Mayfair Chambers, The Terrace, Wellington, phone 724860, 724861, Telex 31487.

Auckland: Four Seasons Plaza, 22 Emily Place, Auckland 1, Phone 3-1116.

ABSTRACTS

HISTOLOGY

Pathological Evaluation of Computed Tomography Images of Lung.
Coddington, R., Mera, S. L., Goddard, P. R. and Bradfield, J. W. B. (1982), *J. Clin. Path.* **35,** 536.

A method is described which allows the features seen in images generated during computed tomography (CT) of lungs previously removed at necropsy to be compared with those seen in corresponding histological sections from the same lungs.

High-Grade Intensification of the End-Product of the Diaminobenzidine Reaction for Peroxidase Histochemistry.
Gallyas, F., Gorcs, T. and Merchenthaler, I. (1982), *J. Histochem. Cytochem.* **30,** 183.

A simple and reliable method is described for the intensification of the end-product of the DAB reaction demonstrating peroxidase activity. After completion of the DAB reaction the preparations are treated with thioglycolic acid followed by a silver bath.

Immunoperoxidase Staining of Legionella Pneumophila.
Boyde, J. F. and McWilliams, E. (1982), *Histopathol.* **6,** 191.

Immunoperoxidase staining has been applied to sections of pneumonic lung from a case of Legionnaires' disease. Specific staining of Legionella pneum. was accomplished with sub-group 1 antiserum. In other sections some organisms stained specifically with rabbit anti-lambda chain serum but not with anti-kappa. This result suggests that the organisms were coated with the patient's IgM specific antibody.

Ten-Minute Silver Stain for Pneumocystis Carinii and Fungi in Tissue Sections.
Musto, L., Flanigan, M. and Elbadawi, A. (1982), *Arch. Pathol. Lab. Med.* **105,** 292.

A simple rapid methenamine silver staining procedure for the demonstration of Pneumocystis carinii and fungi in tissue sections has been developed. The procedure is optimal for both urgent and routine histological diagnosis of diffuse, progressive and life-threatening pulmonary infiltrates, especially in the compromised host, and of fungal lesions in general. The authors claim the results to be superior to Grocott's method.

A Trichrome Stain for Intrahepatic Localization of Hepatitis B Surface Antigen.
Sajjad, S. M. and Ordonez, N. G. (1982), *Arch. Pathol. Lab. Med.* **105,** 298.

A modified trichrome stain was used to detect hepatitis B surface antigen in liver tissue obtained at the time of autopsy. The infected hepatocytes exhibited a selective green metachromasia of their cytoplasm confirmed by immunohistology.

A Celloidin Bag for the Histological Preparation of Cytological Material.
Bussolati, G. (1982), *J. Clin. Path.* **35,** 574.

A method for retaining tissue debris and cell clusters found in cytology specimens during histological processing is described using celloidin bags.

Short Communication—A Simple Method for Incubation of Tissue Sections in Immunohistochemistry.
Ormanns, W. and Pferfer, U. (1981), *Histochemistry,* **72,** 2, 315.

The authors describe the making of an 'incubation chamber' which will guarantee the uniform incubation of tissue sections with minimal reagent use and evaporation. To illustrate its use, a case of alpha-1-antitrypsin deficiency in liver is described.

Evaluation of Colour Transparency Films for Photomicrography of Fluorescent Structures.
Stoddard, F. L. and McCully, M. E. (1981), *Histochemistry,* **73,** 1, 121.

Using several different staining methods on plant structures and examining them with fluorescence microscopy, the merits of 17 commercially available colour transparency films were compared with respect to the intensity of the image obtained and accurate reproduction of the colour.

The Role of Paraldehyde in the Rapid Preparation of Aldehyde Fuchsin.
Nettleton, G. S. (1982), *J. Histochem. Cytochem.* **30,** 175.

Preparation of aldehyde fuchsin normally requires ripening for three to five days. By using a five-fold excess of paraldehyde a fully potent aldehyde fuchsin can be prepared in 24 hours at room temperature.

Recommendations of MRC Supported Seminar on Glycosylated Proteins Held at Massey University on Saturday, 29 May 1982

Principal participants: Dr Richard Larkin, Professor R. Carrell, Professor D. Beaven, Dr B. Linehan, Dr John Baker, Dr Denis Jury, Dr Peter Dunn, Dr Michael Crooke, Dr B. Shirley, Dr M. Lever.

The Seminar was attended by clinicians, biochemists, and laboratory technicians involved in the analysis of glycosylated haemoglobins and other proteins in diabetic subjects. Recommendations were made on the clinical application, method of measurement, and quality control of the measurement of 'glycosylated haemoglobins' but it should be noted that these are short term recommendations only. There was a consensus that the currently available techniques of measurement are less than ideal.

With reference to the chromatographic techniques of measurement of 'glycosylated haemoglobins', the following points were made:

1. The title glycosylated haemoglobin is potentially misleading for the minor haemoglobins, Hb AlA, Ab, AlB and Hb AlC may incorporate other non-glycosylated moieties such as foetal Hb, glutathione Hb occurring on storage, acetylated Hb from Aspirin therapy and Hb linked to acetaldehyde in heavy alcohol drinkers. Further, denatured Hbs resulting from poor sample preparation or storage, may co-chromatograph with the glycosylated Hbs.
2. Sample storage temperatures are crucial. Haemolysates appear to be stable for up to seven days at 5° centigrade and for several months at −70° centigrade or lower. Deep freezing of haemolysates at −20° centigrade causes rapid deterioration of the sample.
3. Analysis with commercial disposable mini-columns appears inadequate and the panel did not support their usage. In particular it was noted that the separation of Hb Al from Hb A_o was poor with lack of robustness in methodology, while the cited coefficient of variation for the tests appear too large to be clinically useful.
4. Analysis of Hb Al with multiple mini-columns is difficult to monitor for quality control. Analysis with high pressure liquid chromatography (HPLC) provides a permanent record of the separations achieved and can be subjected to routine quality controls, but the equipment is expensive and the method is time consuming. Use of single columns with a fraction collector has similar advantages and disadvantages.
5. Analysis with agar gel electrochromatography enables a number of samples to be measured rapidly and easily. This method also lends itself to conventional quality control measures. Further, plates can be prepared in the laboratory to reduce costs. However, the method requires an adequate densitometer which is expensive and further there are some reservations concerning densitometry in general. Iso-electric focusing is an alternative technique, with the ability to separate out a reversible Schiff base moiety from the irreversible ketimine fraction. However, the technique is not suitable for general clinical use.

With reference to colorimetric methods, it was noted that theoretically these are preferable to the chromatographic techniques for they are potentially specific for the hexosamine link, or more particularly fructosamine in this context. Further, these methodologies may be readily automated. However, there are some drawbacks to the methods in use currently:

1. Glucose concentrations in the sample being analysed may influence the result and require removal by prior dialysis of the sample.
2. The analytes being measured by the commonly used technique have not yet been rigorously determined and further work is required in this area.
3. Clinical trial with colorimetric methods to validate the use of the measure are substantially less adequate than for Hb Al or Hb AlC% measures.

In the light of these considerations the Panel made the following recommendations:

Methodology

Short Term:

In the short term a chromatographic system is recommended, either agar gel electrophoresis or alternatively adequate resolution column chromatography.

Long Term:

The Panel encouraged the development of an automated colorimetric technique with identification of the groups being measured.

Standardisation and Quality Control

Short Term:

It was recommended that there should be distribution of samples amongst co-operating laboratories from pooled patient samples. The level of Hb AlC and Hb Al in these samples would be determined by high pressure liquid chromatography.

Long Term:

It was recommended there should be attempts to derive a pure stable preparation of Hb AlC.

Clinical Application

It was recommended that a measure of Hb Al or Hb AlC has application in:

1. Research.
2. Clinical supervision of pregnant diabetic patients and other special groups.
3. To provide an on-going surveillance measure of diabetic patients and other special groups.

To initiate the first stage of a quality control programme a sub-committee was established, comprising Dr Denis Jury, Dr John Baker, Dr Michael Crooke, Dr Brian Linehan (Chairman of the National Technical Committee on Standards and Quality Control in Clinical Laboratories) and Dr Peter Dunn as Convener and Dr Michael Lever.

AWARDS

It is with regret that AMES and EBOS have had to withdraw their award which has been available since 1977. The NZIMLT would like to thank them for their generous support in the past and offer our sincere apprecation to the Sales Managers of AMES and EBOS who made this a valuable and sought after award.

BRANCH NEWS

ANNUAL SEMINAR

MID NORTH ISLAND
PALMERSTON NORTH
13 NOVEMBER 1982

Haemoglobinopathies or Art that a Sickle.
Mr Ross Hewett,
Hutt Hospital.

Inter-laboratory Comparisons of Micro-Bilirubins.
Mr Ian Bardsley,
Hawera Hospital.

Congenital Platelet Abnormality—Case Study.
Mr Gary Milicich,
Wellington Hospital.

The Grapevine and its Product.
Dr Jack Parle,
Regional Director,
Agricultural Research,
M.A.F.

On the map with Polycythaemia.
Mrs Maureen Whineray,
Palmerston North Hospital.

RF by ELISA.
Mr Jim Learmonth,
Palmerston North Hospital.

Immunological Markers in Leukaemia.
Mr Gerry Campbell,
Wellington.

Energy Future for New Zealand.
Dr Ian Watson,
Chemistry Department,
Massey University.

Think Wide.
Mr Ted Norman,
Dannevirke.

A plea for educational help for the Pacific Islands.

FORUM

Dear Sir,

I wish to congratulate the Christchurch Regional Representative, Mr Paul McLeod on the excellent editorial in the August issue of the N.Z.I.M.L.T. In future, when urging fellow workers to join the NZIMLT, I have at my disposal this article to use as ammunition when confronted with the question "what's in it for me?".

Yours sincerely,
Trevor Rollinson, Clinical Biochemistry, Christchurch Hospital, 17/11/82

Dear Sir,

It is with considerable interest that I read Mr Phillip's informative section on proposed training scheme in the latest N.Z.I.M.L.T. journal only to be dismayed and disgusted with the final paragraph.

The attempts by the board to solicit the views of rank and file technologists have been thwarted by time limits. I fail to see how people can consider and comment upon things that affect them when the journal arrives in their possession on 28 September and letters have "to be received by the Board's secretary by the end of September",—i.e. TWO DAYS later. Postage takes longer than that.

Mr Paul McLeod in the last editorial called for more technologist participation—I support that call but I ask "How can we, if we aren't given time to?"

Yours sincerely,
A. G. H. Bennett, Charge Technologist, Haematology Department, Timaru Hospital. 30/9/82.

The Editor wishes to point out that the letter referred to was circulated to all Charge Technologists on 23 July 1982, and was reprinted in the Journal for the information of all members. (Editor).

Dear Sir,

At the Special General Meeting in Christchurch it was pointed out that nursing staff with no medical technology training are performing diagnostic biochemical tests on sophisticated equipment which is simple to use.

In contrast, the Medical Laboratory Technologists Board is embarking on negotiations for a five year course culminating in a degree which is intended to produce generally qualified technologists with a better academic background.

Food for thought, isn't it?

Yours sincerely,
Kevin McLoughlin, Blood Bank CH, 3/12/82.

News from the Hill

Extract from a Speech Read by Dr H. J. H. Hiddlestone Director-General of Health due to the Minister's Absence The Annual Conference of the New Zealand Private Hospitals Association

"Too often, too many people have quite incorrectly stated that the private hospital sector provides an equally efficient service at a much lower cost than our public hospitals."

A truer cost comparison would be to take from the full grants paid from vote: health, the costs of medical staff salaries, pathology and x-ray, accident and emergency, outpatient and day patient, domiciliary and transport services, and a variety of other estimated costs for services or functions not provided by private hospitals.

When these costs are excluded, the public hospital average daily bed cost becomes $88.15.

Professor Ward's report for 1980/81 estimates that private hospital costs per bed day range from $33.35 in medical hospitals to $74.90 in surgical hospitals.

His survey did not provide complete information about larger (over 100-bed) private surgical hospitals.

Using earlier information collected by Professor Ward my department has estimated that these larger private surgical hospital costs could be up to $84 per day.

In general, private surgical hospitals are more expensive than medical, and the bed day cost of surgical hospitals increases with size.

Most private medical hospitals care for geriatric patients and their bed day costs are known to be low.

The cost levels for 1980/81 reported by Professor Ward seem reasonably in line with an estimated income level of $39 per bed day.

This is based on information obtained through monitoring the geriatric hospital special assistance scheme.

Medical beds in public hospitals treat patients, for a variety of conditions, such as burns, cardiac arrest, stroke, and most cancers.

Many such cases are not considered for admission to private hospitals because of the acute and complex nature of their treatment.

It is the sort of story that always gets coverage because bashing public institutions is a national sport of we New Zealanders.

In relation to hospitals however it is time we put that particular ghost to rest once and for all.

When one looks at the level of financial assistance already provided to the private hospital sector, which I referred to earlier tonight, no-one can deny that Government heavily subsidises private hospitals.

In fact, you are much like other sectors of the free enterprise economy in New Zealand in that your levels of income if not your very existence are dependent on taxpayer and Government support.

At my request, my department has recently completed a detailed cost comparison between public and private hospitals.

As I am sure your members will appreciate, this is a very difficult exercise because each sector undertakes different tasks.

Both Professor Ward, who has done some work for your Association on private hospital costs, and the department, consider the most appropriate comparison to be the net bed day costs, after allowances have been made for services provided in the public but not private hospitals, and for the exclusion of medical staff, drug and other costs from private hospital budgets.

The average full cost per day bed in 1980/81 in public hospitals was $129.24.

This figure is calculated by dividing the full amount of grants paid to hospital boards from vote:health by the total number of bed days.

However, this figure gives a false impression as it includes substantial costs of $306.6 million not borne by private hospitals.

Initial diagnosis and treatment of these patients can be both intensive and expensive.

Similar comments can be made about differences between surgical patients.

The smaller private hospitals generally undertake the simpler surgical procedures—varicose veins, tonsillectomies, hernia repairs and ear drum surgery.

Public hospitals undertake cardio-thoracic surgery, neurosurgery, hip replacements and a wide range of surgical interventions to excise cancers and other tumours, plus all urgent accident surgery.

Given the much wider activities and more complicated procedures in public hospitals and consequent need for greater care, the variance between public and private hospital bed costs per day is not excessive.

I support and encourage the private sector but I also support the truth.

The truth is that, on the whole, we have a very efficient hospital system in New Zealand in both the private and the public sectors.

In both we strive constantly to still further improve efficiency and there is room for progress in both.

The two sectors tend to fulfil complementary roles rather than competitive roles however and neither has any right to claim it is uniquely more competent than the other.

NEW ZEALAND INSTITUTE of MEDICAL LABORATORY TECHNOLOGY

39TH ANNUAL SCIENTIFIC MEETING

NEWSLETTER

The 1983 Annual Scientific Meeting will be held in Napier on 18 and 19 August. Your committee is already well on the way planning the various functions, their aim being to make the 1983 Meeting a most memorable one.

The main Conference venue and trade display area will be sited on the Napier Marine Parade with all accommodation and additional venues within a few minutes' walking distance.

As with all scientific meetings, we will be relying heavily on you! In other words, have you thought about presenting a paper? Without a plentiful supply of good papers, the efforts of the committee will be to no avail. An early indication of your intention to present a paper would be appreciated so that a programme can be planned. We have been advised that Commonwealth Serum Laboratories have kindly offered to sponsor the attendance of Professor Peter Issitt, Scientific Director of the South Florida Blood Service, so some good papers in the Immunohaematology field seem to be indicated.

The trades display area promises to be excellent value with twenty-eight companies already indicating their interest in the display area. As we intend morning and afternoon teas being served in the display area and lunches being available in the same building, there will be plenty of time to browse.

The social programme is well in hand, starting with a 'Happy Hour' on the Wednesday evening through to the traditional dine and dance on the last evening. Various trips in and around Napier are being planned, for example a trip around the Hawke's Bay Wine Trail, and will be available if demand is great enough, but more details of that at a later date. For now—start work on those papers and let us know what you are doing. Address all correspondence to the Conference Secretary, C/- Laboratory, Public Hospital, Napier.

Social and Related Community Services
Final Results, Division 93 of the 1980-81 Census of Services

The Department of Statistics has released the final results of Division 93: Social and Related Community Services—which forms part of the Census of Services.

The whole census is included in the series of integrated economic censuses of business activities in New Zealand carried out by the department over a five-yearly cycle and is the first economic Census of Services.

Further information is available from the Executive Officer, Business Censuses, Department of Statistics, Private Bag, Auckland (phone 32-245).

Division 93 Industry Statistics—Census of Services 1980/81

NZSIC Reference Number	Industry Description	Establishments etc.	Working Proprietors and Partners		Paid Employees			Total Income adjusted for Stocks	Net Profit
			Number	Salaries	Number	Salaries and Wages	Total Expenditure		
		No.	No.	$(000)	No.	$(000)	$(000)	$(000)	$(000)
93317	Public and Private Hospitals	378	65	935	63,545	700,239	946,852	934,167	−13,621
93319	Other Health Services	417	158	427	4,559	43,619	58,062	59,649	1,160
93321	Medical Laboratories	126	44	244	1,100	10,075	18,012	23,334	5,079

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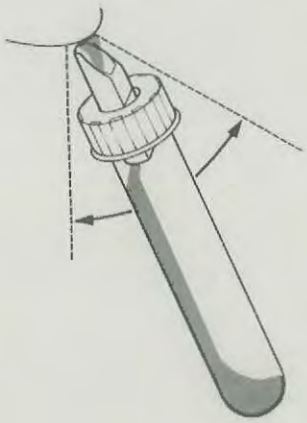
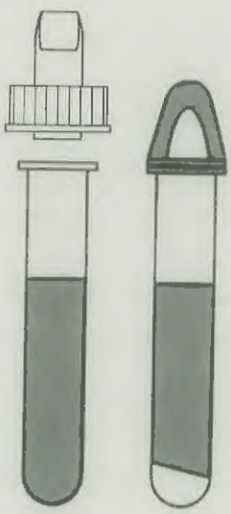
5. Avoid milking of puncture site.

Collection Procedure

Hold MICROTAINER Tube at an angle below horizontal; vent hole upward. Touch tip of FloTop Collector to *underside* of drop, as shown. Do *not* touch tip to the middle of the drop. Blood will flow freely through the FloTop Collector and down the tube wall.





Cap.
Serum tubes.
Remove FloTop Collector from MICROTAINER Tube and discard. Replace with plug.

Send specimen to laboratory.

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WANTED: Permanent Laboratory Position. Registered U.S. Medical Technologist desires position. NZMTB will allow Part III examination to be taken after 1 year for limited registration. Have had 3 years experience in Microbiology, including supervisory experience. Qualified to accept a position in other areas. Resume and references available. Phone 534-4846 Auckland, or write Beverly Klaty, 11 Endymion Place, Half Moon Bay, Auckland.

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1. Lee, C. L. and Davidson, I: Serologic Tests for Infectious Mononucleosis, ASCP Commission on Continuing Education, 1972.
2. Lee, C. L: Spot Test for Infectious Mononucleosis, Bull of Path, 1968

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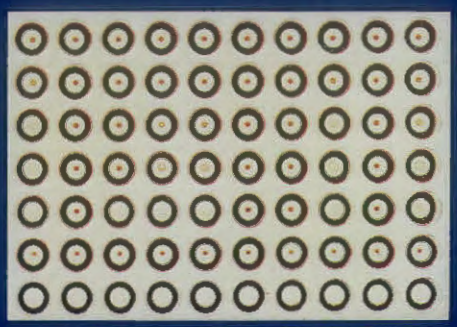

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