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You are invited to submit 15-20 minute papers and/or poster presentations for the above meeting. Abstracts must be typed in single space type in less than 250 words. These must be free of grammatical and typographical errors. The original and two photocopies should be submitted.

PLEASE COMPLETE THE FOLLOWING QUESTIONNAIRE:

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Presenter (Title, First Name, Surname) .....

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PLEASE FORWARD YOUR ABSTRACT
NO LATER THAN 30 JUNE 1993 TO:

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Ngaio Marsh Conference Centre
University of Canterbury
25th — 27th August 1993

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Tuesday 24th August:

WORKSHOPS:
NZIMLS workshop for Moderators and Examiners

Amersham User's Meeting
DNA Antibody Workshop — Immunology

SOCIAL:
Wine and Cheese Evening

Wednesday 25th August:

OPENING CEREMONY:
NZIMLS Annual General Meeting
Forum: CHE: Changing Health Environment
Dr B. Layton (Christchurch CHE)
Mr B. Bullen (Composite CHE)
Mr T. Czura (S.G.S)
Mr G. Simpson (Cardinal)

SOCIAL:
Pizza Evening with 70's Disco

Thursday 26th August:

CONCURRENT FORUMS:

BIOCHEMISTRY:
Proferred papers for the Boehringer Medal
Update in Lipid Testing
Renal Function Tests Reviewed
Recent Advances in Automated Immunoassay

IMMUNOLOGY:
Clinical Interpretation of Immunological Tests
Quality Control of Immunological Techniques
Immunology of Pregnancy and Reproduction
Discussion on Allergic Reactivities

MICROBIOLOGY:
Current Status of Automation in the Clinical Microbiology Laboratory
Mycobacteria: Interfacing of Medical and Veterinary Technologies
General Microbiology: Proferred Papers
Renal Microbiology

N.Z.I.M.L.S. 48TH ANNUAL SCIENTIFIC MEETING

PRELIMINARY PROGRAMME:

HAEMATOLOGY:
Main Theme: Platelets — covering all aspects including morphology, function and count. Papers on this and other topics are invited.

TRANSFUSION MEDICINE:
Emergency Transfusion
Rh Mosaics
Quality Assurance
Hepatitis C: One Year in Retrospect

HISTOLOGY/CYTOLOGY:
Forensic Pathology
Renal Biopsies
Problems with Immunohistochemistry
Cytology - Some Case Studies

SOCIAL: Conference Dinner: Theme Rock and Roll

Friday 27th August:

GENERAL FORUMS:

MOLECULAR BIOLOGY:
Basic Introduction to Molecular Biological Techniques
Forensic Science Case Studies
Molecular Biology Approaches to Infectious Diseases of Man and Animals
Genetic Basis of Disease
The Molecular Biology of Virology

QUALITY ASSURANCE AND REGULATORY AFFAIRS:
Quality Assurance Programmes
TELARC Registration
Technologist Board Regulations and Registration

CLOSING CEREMONY

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Original Articles

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From Vol. 36 No. 1 all papers published will be in the form known as "Vancouver Style" or Uniform Requirements for Manuscripts submitted to Biomedical Journals. Full details may be found in the New Zealand Journal of Medical Laboratory Science, Vol. 45, No. 4, page 108 to 111 or from the Editor.

Intending contributors should submit their material to the Editor, M. Gillies, Microbiology Laboratory, Auckland Hospital, Auckland, 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 issue is the first of the month prior to the month of publication.

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Abstract writing for Scientific Meetings

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Introduction

Upon reviewing the abstracts received for presentation at the 47th Annual Scientific meeting of the NZIMLS at Wellington 1992, it was noted by the authors that not only was the scientific quality of some abstracts sub standard, but many did not conform to the guidelines published in the call for abstracts (1). The call for abstracts for this conference specified that they should contain (a) a concise statement of the study's objective (b) a brief statement of the methods used (c) a summary of the results and (d) the conclusion. Typing instructions were included and additionally intending authors were asked not to state in their abstract that "The results will be discussed".

Given that various sessions at the Conference are concurrent, conference delegates generally read the abstracts to determine which of the papers being presented are potentially worth attending. Therefore it is paramount that as professionals we should prepare abstracts for our scientific meetings to the same high standard as is required for papers submitted for publication to peer-reviewed biomedical journals (2). It is the purpose of this paper to present and discuss various "do and do nots" when submitting abstracts for presentation at scientific meetings. It is hoped that this will improve the standard of abstracts submitted to future conferences, which ultimately is of benefit for our profession.

Abstract writing. Do and Do Nots

Do Follow fully the instructions for preparation of abstracts as required by the Conference organisers.

Do Use statistics if appropriate and describe them briefly.

Do Use S.I. units and their correct abbreviations. For instance 10 mM should be written as 10 mmol/L.

Do Economise on words. Remember that you generally have to present your information in about 250-300 words. Certain phrases can often be replaced by a single word, ie "Which is shown to be" can be replaced by "is".

Do Check your spelling.

Do Explain in full the first time abbreviations are used in the text.

Do Get your colleagues to read and comment on your abstract.

Do Number your references in the text and use the Vancouver (2) system in listing at the end of the abstract.

Do Acknowlege significant support for the study if appropriate.

Do not Use abbreviations in the title.

Do not Use too long an opening paragraph.

Do not Make the abstract too long or too short.

Do not State unnecessary facts.

Do not Make conclusions which are not supported by the results and/or statistical analysis.

Do not Make claims without supporting data.

Do not Use the two following statements:
(a) data will be presented
(b) results will be discussed.

Discussion:

Good abstracts follow a specific layout and are generally structured in paragraphs which briefly describe:

(a) the study's objective(s).
(b) the method(s) used, inclusive of statistical analysis.
(c) the main results obtained, with statistical significance if appropriate.
(d) the main conclusion(s) drawn from the results obtained keeping in mind the study's main objective(s).

Additional features of a good abstract are novelty of the study and potential applicability. Briefly highlighting any problems or difficulties encountered during the study can alert colleagues engaged in similar work. Remember that well written abstracts are of actual use to other members of our profession, many of whom are unable to attend the scientific meeting. If in doubt or inexperienced consult a colleague or other health professional with expertise in writing scientific abstracts.

These guidelines are appropriate for studies which have a defined objective, with obtained results supporting the main conclusion. A slightly different format is generally appropriate for case studies and reviews. However a majority of the above stated guidelines and the "Do and Do Nots" in general also apply to these types of abstracts. When describing case studies a brief paragraph stating the type of case study is generally followed by the main laboratory and/or clinical findings relevant to the study. It is also worthwhile presenting the results on completion of the case and/or treatment thereof. For review abstracts the main subject of the review is briefly described followed by the salient facts of the review topic.

It is not good practice to include speculative or controversial statements unless they can be reasonably argued by supportive data. Again we would like to reiterate one of the major Do Nots, namely the use of the statements "data will be presented" and "results will be discussed". These statements do not have a place in a good abstract and are of no use to our colleagues unable to attend the presentation. It is hoped that this brief article is of use when preparing an abstract for a scientific meeting. Additional to the improvement of abstracts appearing in the scientific meeting programme, it will ease the burden of the scientific meeting organisers, for instance in having to ask authors to rewrite their abstracts.

Acknowledgement:

The authors wish to thank Mrs Maureen Gordon for typing the manuscruipt.

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1. Call for abstracts. NZIMLS 47th Annual Scientific Meeting, Wellington 1992. *NZ J Med Lab Science* 1992; **46**(2):63.
2. International Committee of Medical Journal Editors. Uniform requirements for manuscripts submitted to biomedical journals. *Br Med J.* 1991; **302:** 338-41.

NZJ Med Lab Science 1993; 47(1) : 7-10.

Evaluation of laboratory methods for the determination of methicillin susceptibility in New Zealand isolates of *Staphylococcus aureus.*

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Abstract

One hundred and twenty-two isolates of *Staphylococcus aureus* were tested for oxacillin and methicillin susceptibility by the reference method (oxacillin microbroth dilution), and methicillin agar dilution, agar screen and disc diffusion methods. Compared with the oxacillin microbroth test, methicillin agar dilution tests were both senstitive and specific. Methicillin agar dilution and agar screens were more sensitive and specific than the standard NCCLS disc diffusion method. Disc diffusion methods using NCCLS protocols were more sensitive and specific than disc diffusion using Stoke's method. The addition of 4% NaCl to Mueller-Hinton agar increased the sensitivity of the NCCLS disc diffusion tests, but generally decreased the specificity. Our study showed that supplementation with salt, incubation temperature, and type of inoculum affected the sensitivity and specificity of the test.

Keywords

MRSA, methicillin-resistant *Staphylococcus aureus*, methicillin susceptibility, methods.

Introduction

Methicillin resistance in *Staphylococcus aureus* is most often heterogenous and this can make its detection difficult in the clinical laboratory. Detection of methicillin resistance is improved by the addition of salt to the media (1), incubation at 30°C (2), the use of a heavier inoculum (3), or the use of a direct inoculum prepared from an overnight plate (4). The microbroth dilution method (5) has been recommended by the National Committee for Clinical Laboratory Standards (NCCLS) for the detection of methicillin-resistant *S. aureus* (MRSA) and is widely used as a reference method.

In New Zealand, a variety of methods are used to test for methicillin resistance. In contrast to other countries, a large proportion of the MRSA isolates in New Zealand have low-level methicillin MICs, 8-32 mg/L when tested by the NZCDC method (6), and are not usually multiply-resistant.

We undertook the present study to evaluate several methods recommended for the detection of methicillin resistance. We also investigated the effect of the addition of salt to the media, incubation at either 30°C or 35°C and the use of direct inocula versus log-phase inocula. In particular, we wanted to ascertain the best method for detecting MRSA in New Zealand. We selected the oxacillin microbroth dilution test as the reference test and compared all other methods with it.

Materials and Methods

Bacterial strains

One hundred and twenty-two clinical isolates of *S. aureus* referred to NZCDC between 1987 and 1990 were tested for methicillin susceptibility. We selected isolates with a variety of phage typing patterns and a range of methicillin MIC's, as determined by the NZCDC method.

Antimicrobial agents and media

The oxacillin and methicillin reference powders were kindly supplied by SmithKline Beecham (NZ) Ltd. Oxacillin and methicillin discs were obtained from Oxoid (NZ) Ltd.

Mueller-Hinton (M-H) broth and agar were obtained from Difco Laboratories, Isosensitest agar (ISA) was obtained from Oxoid (NZ) Ltd. The same batch of media was used for all the tests.

Susceptibility tests

All isolates were tested by the following standard methods and variations.

Microbroth dilution method: Oxacillin microbroth MIC's were determined, in duplicate, as recommended by NCCLS (5) using cation-supplemented M-H broth with 2% NaCl, an inoculum of 5 x 10^5 cfu/ml prepared from overnight plates (direct inoculum) and incubation at 35°C for 24h. Isolates with oxacillin MIC $\geq$ 4 mg/L were considered to be resistant.

Agar dilution method: Methicillin MIC's were determined by the NZCDC method (6) using M-H agar, log-phase inoculum (inoculum grown at 37°C for 4-5h) of 10^4 cfu and incubation at 30°C for 24h. In addition, methicillin MIC's were also determined on M-H agar with 4% added NaCl, using direct inoculum and by incubating at 35°C. All isolates were tested in duplicate or triplicate. Isolates with methicillin MIC $\geq$ 8 mg/L were considered to be resistant.

Agar screen method: Agar screens (4) were performed in duplicate or triplicate with M-H agar supplemented with 4% NaCl and oxacillin 6 mg/L or methicillin 10 mg/L. A direct inoculum of 10^4 cfu per spot was replicated onto the agar screen plates. In addition, agar screen tests were also performed with a log-phase inoculum. Isolates that grew on the agar screen plates after 24 h or 48 h incubation at 35°C were considered to be resistant.

Standard NCCLS disc diffusion method and variations: The standard NCCLS disc diffusion test (7) was performed using oxacillin 1 ug and methicillin 5 ug discs, M-H agar, incubation at 35°C and a direct inoculum adjusted to 10^8 cfu/ml. Variations of the NCCLS disc test were carried out by adding 4% NaCl to the media, incubating at 30°C and using a log-phase inoculum. Zone diameters were interpreted as recommended by NCCLS (7).

Stoke's disc diffusion method: Stoke's disc diffusion tests (8) were performed using methicillin 5 ug and methicillin 10 ug discs on ISA with an inoculum which gave semi-confluent growth. Tests were carried out using direct and log-phase inocula, ISA with and without 5% NaCl, and incubation at 30°C and 35°C. Results were interpreted by comparison with a sensitive control, *S. aureus* (NCTC 6571). Isolates were considered to be sensitive if the zone size (edge of disc to edge of zone) was equal to, wider than, or not more than 3mm smaller than the control; resistant if the zone size was 3mm or less; and moderately sensitive if the zone size was greater than 3mm, but smaller than the control by more than 3mm.

Data Analysis

The sensitivity of the test was defined as the number of resistant isolates obtained by the test divided by the number of resistant isolates obtained by the oxacillin microbroth test.

The specificity of the test was defined as the number of sensitive isolates obtained by the test divided by the number of sensitive isolates obtained by the oxacillin microbroth test.

Results

Of the 122 isolates, 94 isolates were classified as MRSA and 28 as MSSA (methicillin-sensitive *S. aureus*) by the oxacillin microbroth dilution method.

Agar dilution and agar screen tests

The specificity of agar dilution methods was 100% (Table 1). The sensitivities ranged from 98—100% except for the

Table 1: Methicillin susceptibility of 94 oxacillin-resistant and 28 oxacillin-senstitive *S. aureus* isolates determined by methicillin agar dilution and agar screen methods.

Media and Conditions	Oxacillin Resistant* (Number = 94)		Oxacillin Sensitive* (Number = 28)		Sensitivity %	Specificity %
	R	S	R	S		
Methicillin agar dilution						
MH 30° L (NZCDC)	92	2		28	98	100
35° L	78	16		28	83	100
30° D	92	2		28	98	100
35° D	92	2		28	98	100
MHS 30° L	92	2		28	98	100
35° L	92	2		28	98	100
30° D	94			28	100	100
35° D	92	2		28	98	100
Oxacillin agar screen						
D 24 h	87	7		28	93	100
L 24 h	79	15		28	84	100
D 48 h	91	3		28	97	100
L 48 h	82	12		28	87	100
Methicillin agar screen						
D 24 h	90	4		28	96	100
L 24 h	81	13		28	86	100
D 48 h	92	2		28	98	100
L 48 h	84	10		28	89	100

Key to Tables 1, 2 and 3:

* = Classification by oxacillin microbroth method
R = Resistant by test
I = Intermediate by test
S = Sensitive by test
MS = Moderately sensitive by test
NG = No growth or poor growth
ISA = Isosensitest agar
ISAS = Isosensitest agar + 5% NaCl
D = Direct inoculum
L = Log-phase inoculum
MH = Mueller Hinton Agar
MHS = Mueller Hinton + 4% NaCl
** = No growth of one isolate (same isolate)

method using M-H agar, log-phase inoculum and incubation at 35°C which was markedly less sensitive. The NZCDC method detected 98% of the MRSA and 100% of the MSSA isolates. The agar dilution method using M-H agar with added 4% NaCl, direct inoculum and incubation at 30°C correctly identified all the MRSA and MSSA isolates.

The specificity of agar screens was 100% but agar screens were less sensitive than agar dilution tests (Table 1). The use of direct inoculum and 48h incubation increased the sensitivity of agar screen tests. Methicillin agar screens with direct inocula detected 96% and 98% of the MRSA isolates after 24 h and 48 h respectively.

Standard NCCLS disc diffusion tests and variations

The standard NCCLS disc test (Table 2) detected 91% of the MRSA and 93% of the MSSA isolates. Sensitivity was consistently higher with the oxacillin 1 ug disc but specificity was generally higher with the methicillin 5 ug disc.

The addition of 4% NaCl improved the sensitivity of tests using both oxacillin and methicillin discs but usually at the expense of their specificity. This effect of 4% NaCl was more pronounced when the incubation temperature was 35°C. Incubation of tests at 30°C increased the sensitivity of tests carried out on media without added 4% NaCl. The type of inoculum had little effect on the sensitivities and specificities of most tests.

Stoke's disc diffusion tests

The sensitivity of disc diffusion tests using the Stoke's method (Table 3) was much lower than for the other methods. This was due to the classification of a number of oxacillin-resistant isolates (from six to 54 isolates) as moderately sensitive. Three isolates did not grow or grew poorly on the ISA when tests were incubated at 30°C.

Tests using methicillin 5 ug discs were more sensitive while tests using methicillin 10 ug discs were more specific. The addition of 4% NaCl decreased the specificity of all tests and increased the sensitivity of tests incubated at 35°C. A direct inoculum increased the sensitivity of tests carried out with methicillin 10 ug discs and incubated at 30°C.

Discussion

The current NCCLS recommended tests (9) for the detection of methicillin resistance in *S. aureus* are the microbroth dilution test using either oxacillin or methicillin and 2% NaCl supplemented M-H broth, the disc diffusion test using either oxacillin 1 ug or methicillin 5 ug discs on unsupplemented M-H agar, and the agar screen test using 4% NaCl supplemented M-H agar and 6 mg/L oxacillin or 10 mg/L methicillin.

Our study showed that, compared with the oxacillin microbroth dilution method, methicillin agar dilution tests were the most sensitive and specific of all the methods tested. Methicillin agar dilution on M-H agar with added 4% NaCl, direct inoculum and incubation at 30°C correlated completely with the oxacillin microbroth dilution test results. It is notable that this test uses several of the special conditions

Table 2: Methicillin susceptibility of 94 oxacillin-resistant and 28 oxacillin-senstitive *S. aureus* isolates determined by the standard NCCLS disc diffusion method and its variations.

Media and Conditions	Oxacillin Resistant* (Number = 94)			Oxacillin Sensitive* (Number = 28)			Sensitivity %	Specificity %
	R	I	S	R	I	S		
Oxacillin 1 ug disc								
MH 35° D	86	3	5		2	26	91	93
(std NCCLS method) 35° L	85	1	8		1	27	90	96
30° D**	90	1	2		1	27	96	96
30° L**	89		4			28	95	100
MHS 35° D	94			4	2	22	100	79
35° L	93	1		2	2	24	99	86
30° D	94				2	26	100	93
30° L	94			1	1	26	100	93
Methicillin 5 ug disc								
MH 35° D	69	18	7			28	73	100
(std NCCLS method) 35° L	63	22	9		1	27	67	96
30° D**	88	1	4			28	94	100
30° L**	85	4	4			28	90	100
MHS 35° D	89	5			2	26	95	93
35° L	91	3			2	26	97	93
30° D	89	5				28	95	100
30° L	90	3	1			28	96	100

Table 3: Methicillin susceptibility of 94 oxacillin-resistant and 28 oxacillin-senstitive *S. aureus* isolates determined by Stoke's disc diffusion methods.

Media and Conditions	Oxacillin Resistant* (Number = 94)				Oxacillin Sensitive* (Number = 28)				Sensitivity %	Specificity %
	R	MS	S	NG	R	MS	S	NG		
Methicillin 5 ug disc										
ISA 35° D	72	22				1	2	25	77	89
35° L	71	23				2	26		76	93
30° D	81	10		3		1	27		86	96
30° L	80	11		3		2	26		85	93
ISAS 35° D	88	6			2	9	17		94	61
35° L	87	7			2	8	18		93	64
30° D	79	13		2		6	22		84	79
30° L	74	18		2		11	17		79	61
Methicillin 10 ug disc										
ISA 35° D	41	52	1			2	26		44	93
35° L	39	54	1				28		41	100
30° D	62	29		3		1	27		66	96
30° L	53	38		3		1	27		56	96
ISAS 35° D	66	28				6	22		70	79
35° L	55	39				6	22		59	79
30° D	64	28		2		4	24		68	86
30° L	53	39		2		6	22		56	79

known to enhance sensitivity with no loss of specificity. As expected, the agar dilution with no special conditions, i.e. unsupplemented medium, log-phase inoculum and incubation at 35°C, was markedly less sensitive.

The agar screen test has been shown to have good specificity (10). Likewise, we found agar screen tests to be very specific. In contrast, another study (11) reported agar screens as sensitive but not specific. We found that agar screens were less sensitive than methicillin agar dilution tests. However, our agar screens were carried out following the earlier recommendations of Thornsberry and McDougal (4). The current recommendations exclude 48 hour incubation and a heavier inoculum is recommended.

Even though the disc diffusion tests appeared less sensitive than the agar dilution tests in our study, it should be noted that there was an inherent unfairness in comparing the disc diffusion tests with the oxacillin microbroth test. The microbroth method only divides strains into resistant and sensitive categories whereas the Stoke's disc diffusion method includes a moderately susceptible category and the NCCLS disc diffusion method an intermediate category.

The standard NCCLS disc diffusion tests and its variations appeared more sensitive than tests using Stoke's method. It is notable that up to 54 isolates were classified as moderately sensitive by the Stoke's method. As previously observed (12), Isosensitest agar did not support the growth of all the strains.

Media supplementation with NaCl increased the sensitivity and decreased the specificity of all disc diffusion tests incubated at 35°C. Similarly, incubation at 30°C increased the sensitivities and decreased the specificity of all disc diffusion tests performed without added NaCl. In our study the standard NCCLS disc diffusion test only detected 91% of the MRSA and 93% of the MSSA isolates. The sensitivity of the standard NCCLS disc diffusion test increased to 100% when 4% NaCl supplemented M-H agar was used. However, there was a decrease in the specificity of the test. This was in contrast to the agar dilution method where the combination of NaCl supplementation, incubation at 30°C and direct inoculum enhanced the sensitivity with no loss of specificity.

We found that the standard NCCLS disc test and its variations using 5 ug methicillin discs were more specific while tests using 1 ug oxacillin discs were more sensitive. In contrast, methicillin and oxacillin have been shown to be equally effective in the classification of MRSA using microbroth dilution and agar dilution tests (10,11).

All the NCCLS recommended tests specify the use of a direct inoculum. We found that tests using a direct inoculum were more sensitive for agar screen tests and for Stoke's disc tests carried out with methicillin 10 ug discs and incubated at 30°C. For all the other tests, the type of inoculum used did not have much effect.

Recently, several studies (13,14 and 15) have compared conventional sensitivity methods for detecting methicillin resistance with methods for the detection of penicillin-binding protein (PBP) 2a, which is uniquely associated with methicillin resistance, and/or the mec-specific gene which codes for production of PBP 2a. All these studies have shown that methods of probing for the mec-specific gene or detecting PBP 2a are more accurate than conventional sensitivity tests in predicting methicillin resistance.

However, as methods for PBP 2a detection and the probe methods are not easily performed in the setting of most clinical laboratories, conventional sensitivity methods still have to be relied upon for predicting methicillin resistance. A combination of tests as recommended by the NCCLS could provide the best options.

Our study showed that for New Zealand MRSA isolates, the methicillin agar dilution test with added salt correlated completely with the oxacillin microbroth test. Our results also showed that supplementation with salt, incubation temperature and type of inoculum affect the sensitivity and specificity of the test. The choice of conditions to use with any of the tests depends on whether sensitivity or specificity is considered more important.

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NZIMLS ANNUAL STAFFING SURVEY

Medical Laboratory Technologists

Currently employed

	1984	1985	1986	1987	1988	1989	1990	1991	1992
Clinical Biochemistry	174	187	186	187	187	175	208	182	202
Microbiology	164	168	172	176	186	189	204	183	206
Haematology	160	160	163	168	176	174	180	162	167
Immunohaematology	86	90	92	97	102	96	105	101	120
Histology	22	24	24	24	28	26	29	34	35
Cytology	6.0	5.2	7.2	5.7	7.8	9.5	22	26.6	23.5
Nuclear Medicine	6.2	8.5	8.0	5.8	9.0	7.0	8.4	9.2	12.2
Immunology	23	22	28	22	21	30	31	34	38
Cytogenetics	5.5	7.5	6.5	7.5	8.0	6.4	5.8	12.6	14.7
Virology	1.0	2.0	6.0	4.5	6.5	10	12	13.5	13.6
Administration (full time)	37	34	39	34	33	33	30	29	39
On rotation	46	41	55	41	44	40	31	31	34
Other	4.5	7.3	2.4	3.0	11	7.8	13	8.6	9.7
TOTAL	735.2	756.5	789.1	775.5	819.3	803.7	879.2	826.5	914.7

Medical Laboratory Assistants

Currently employed

	1984	1985	1986	1987	1988	1989	1990	1991	1992
Clinical Biochemistry	188	193	183	169	174	177	154	133	135
Microbiology	165	186	168	152	188	176	185	156	150
Haematology	142	145	143	117	112	118	120	92	85
Immunohaematology	101	118	118	114	112	100	98	83	87
Histology	78	77	85	76	96	76	74	56	66
Cytology	60	32	36	40	35	56	59	49	56
Nuclear Medicine	16.0	12.5	16.8	11	13	9	4	3.2	5
Immunology	41	32	42	31	48	46	42	31	29
Cytogenetics	5.0	4.0	7.5	5.5	13	3.5	3.5	1.2	2
Virology	5.6	7.0	7.0	8.0	6.5	5.5	6.5	6.5	1
Blood collection	87	96	91	91	75	77	71	68	108
On rotation	56	44	51	56	67	64	28	40	26
Other	24	31	44	49	49	66	50	47	52
TOTAL	948.6	977.5	992.3	919.5	988.5	974.0	895	765.9	802

Students (as at 1st January 1993)

		1993
Auckland Technical Institute (NDMLS)	Year 2	10
	Year 3	20
	Year 4	31
Massey University (BMLS)	Year 2	30
	Year 3	20
Otago University (BMLSc)	Year 2	40
	Year 3	30

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NZJ Med Lab Science 1993; 47(1) : 14-16.

Application of the Polymerase Chain Reaction to the diagnostic laboratory.

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Introduction

Since the polymerase chain reaction (PCR) was first introduced in 1985 (1) it has revolutionised the analysis of DNA. In New Zealand, PCR is in routine use in research laboratories where it has allowed very rapid isolation of tiny DNA fragments of interest and spawned a number of publications (2-6). All over the world PCR technology is increasingly being applied to routine clinical diagnosis of disease (7) where it has enormous potential because it is rapid, inexpensive, and simple to perform. PCR technology is not, however, without its problems. New Zealand is a little slower than the rest of the first world to incorporate PCR into clinical diagnosis, so we are now in the unique position of being able to assess and understand mistakes and problems made in other centres before attempts are made here for a full-scale launch into routine PCR. Our laboratory has been using PCR in research and diagnosis since 1989, and has established PCR assays for detection of a number of important human conditions, including Cystic Fibrosis (CF) and Hepatitis C Virus (HCV). In this article we will briefly explain how PCR works and give practical guidelines for its application to routine analysis, referring to our experience in the development and application of diagnostic PCR assays.

What is the Polymerase Chain Reaction?

The PCR is an enzymatic amplification process which yields large amounts of a piece of DNA of interest, from only a small amount of often very crude starting DNA. In a typical PCR a small quantity (10-100ng) of double-stranded target DNA is mixed with all four deoxyribonucleotides (the building blocks of DNA), two single stranded synthetic DNA primers, a thermostable DNA polymerase and a suitable buffer. The key to the reaction lies in the design of the primers which both define the target and lead to its exponential amplification. The concept of amplification is very simple: target DNA is split into its two single strands by heating (to approximately 95°C), the primers are allowed to anneal to the target by cooling to an optimal temperature (usually in the range of 45-65°C) and each primer is extended by the thermostable polymerase which adds nucleotides to only one end of the primer (the 3' terminus), the sequence being determined by the sequence in the target strand. Thus the elongated primer is formed into a new strand of DNA. Since this process has occurred with the other primer annealed to its target strand, the number of targets has effectively doubled. Another round of heating and cooling sees the target double in number again. Each PCR cycle thus consists of three components: denaturation, annealing and extension. The cycle is repeated (usually 20 to 30 times) and results in a logarithmic amplification of the sequence bounded by the primers.

In a single PCR cycling procedure, three main phases occur (8): Screening, in the early cycles, massive amplification in the middle cycles, and template self annealing in the late cycles. The first few cycles of PCR, the screening phase, are very important as they form the amplification products upon which the massive amplification takes place. In the late cycles the concentration of amplified product increases so much that during annealing single stranded amplified products compete with primer for target and so decrease the amplification potential of the reaction.

The sequence specificity of primer annealing during the screening phase can, by a high annealing temperature, be made sufficiently stringent that very small differences in target sequence can be discriminated between. In Allele Specific PCR (AS-PCR) (also known as the Amplification Refractory Mutation System (ARMS)), one primer of the pair is designed so that it hybridises to only a specific sequence in the target DNA, but not to sequences which differ by even a single nucleotide, as might occur in allelic variation of a gene. Amplification occurs only when the primer is perfectly annealed so the presence of an amplified PCR product indicates the presence of the targeted allele.

The AS-PCR approach has been used in our laboratory as the basis of an assay to detect a clutch of mutations which cause cystic fibrosis (CF). Using AS-PCR, amplification will occur only if the targeted allele is present. CF is a disease characterised by the secretion of thick sticky mucous in the lung and intestine. It invariably leads to chronic chest infection and digestive problems and is usually fatal by early adulthood. It is now known that defects in a cell membrane protein (the cystic fibrosis transmembrane conductance regulator) which controls salt levels in cells in these tissues leads to CF. These defects are due to mutations in the genetic blueprint for this protein, and are inherited from generation to generation. An ongoing worldwide study has identified over 200 different mutations, each of which leads to a defective protein. In New Zealand, as in most parts of the world, one particular mutation (a three base deletion) is the most common (80%) and is easily detected due to the size difference between the mutated and normal amplification products. Several other mutations, however, frequently occur (four more account for another 10% of NZ CF mutations). Using AS-PCR, we can detect any of these four mutations in a single multiplex PCR reaction while also amplifying the most common mutation. Multiplex reactions involve addition of a number of different primer pairs in the same reaction tube and save a great deal of time in testing for conditions like CF. However, they require careful development in order to avoid undesirable interactions between amplification products or primers. Different combinations of primer sequence, concentration and size of amplified product may need to be tested before a robust system is established. In addition, AS-primers may not work well on a wide range of sample types. We regularly receive samples from neonates in the form of Guthrie spots (dry spots of blood on blotting paper) and these are not suitable for AS-PCR.

If the specificity of the first few PCR cycles is poor, non specific amplification products can result. Such artifacts can result from too low an annealing temperature or non-specific hybridisation of primer to template at temperatures where thermostable DNA polymerases have partial activity (30-60°C). These non specific products can subsequently be massively amplified and occasionally be amplified in preference to the true target sequence. It is therefore very important for any PCR to ensure high specificity in these early cycles. A number of techniques, including Hot Start PCR (9) and Booster PCR (10) have been developed in order to optimise the specificity of the first few PCR cycles. These techniques both involve exclusion of a crucial reagent (primers or thermostable DNA polymerase) until after the mixture has reached a temperature of 70°C or more. In our laboratory we use a Hot Start procedure which involves assembling all reagents in a tube, except the thermostable polymerase, followed by heating the reaction to 98°C for 10 minutes to guarantee complete denaturation of the target. The reaction is then cooled to the annealing temperature, a thermostable polymerase added and cycling allowed to continue as usual. This procedure allows elimination of some artifact bands as well as allowing amplification of otherwise unamplifiable poor quality DNA samples. This Hot Start procedure is essential for the successful amplification of the main CF mutation from dried blood spots.

The strength of PCR is that it allows massive amplification of a very small amount of template. As described this strength can be a problem if non-specific primer annealing occurs in the first few cycles. It can also be a significant problem for contamination of reactions, since the tiniest amount of contaminating target can be amplified and give spurious results. Because of the amplification potential of PCR, contamination is a considerable problem in the diagnostic laboratory where large numbers of samples are being amplified with the same set/s of primers. The avoidance of PCR contamination requires careful planning, meticulous laboratory practice and high standards of staff training.

Avoidance of contamination

There are three sources of contamination of PCR: DNA from the operator, DNA from other samples in the same run (carryover) and previously amplified PCR products. Good laboratory practice will virtually eliminate the possibility of contamination from the operator, so it is previously amplified PCR products and, to a lesser degree, DNA from other samples in the same run which comprise the greatest risk for contamination of future reactions (11). For this reason PCR assembly and PCR product handling procedures *must* be physically separated. We have separate laboratories for these purposes. In the pre-PCR room DNA extractions and PCR reactions are set up, and the PCR machines themselves are stored. Post-PCR work is carried out in our main laboratory and is where electrophoresis and all manipulation of PCR products occur. In the pre-PCR room we have reagents, pipettes, freezers and equipment which are dedicated to pre-PCR work only. All reagents used for PCR are stored in frozen aliquots, and only one aliquot is used at a time. None of these reagents or equipment has ever been in the room where analysis of PCR products takes place and we have a very strict rule that PCR products must never enter the room. PCR reactions are assembled in a Class II hood with the operator wearing gloves. Because so little target is required to contaminate future reactions it is essential that dedicated pipettes which have never been used for handling of PCR products are used. The tiniest quantity of PCR product-containing aerosol in the barrel of a pipette is sufficient to cause contamination. If pipettes are accidentally taken into the post PCR room they must not be allowed to return until they have been thoroughly decontaminated. This can be done by treatment with 0.1M HNO_3, 2% HOC1, or UV illumination for 30 minutes (although some plastics may not tolerate these conditions). Positive displacement pipettes are used by many in assembly of PCR reactions as these cannot be physically contaminated. Although each positive displacement tip is considerably more expensive than standard pipette tips, they are a good investment for a laboratory starting out with PCR. Our experience with both types of pipette has been that with the described precautions standard pipettes can be safely used. In any case, it is essential that a control reaction (which contains water instead of template DNA) be included in every PCR run to monitor for the presence of contamination. If contamination is detected, any suspect aliquots of reagent can be replaced with fresh ones.

The other important source of contamination, DNA from other samples in the same run or 'carryover' can be avoided by the way in which the PCR reactions are assembled. Our standard procedure is to make a master premix in a separate tube into which all PCR reagents, excluding template DNA, are added. A single drop of mineral oil is added to each reaction tube, followed by aliquots of the master premix to reach reaction tube. Sample template is then carefully added to each of the tubes, and an equal volume of water added to the final tube as a negative control. The tubes are then briefly microfuged to bring the reaction through the oil, then amplified by thermocycling. Using this procedure we have never had carry over problems.

A simple way of ensuring PCR products synthesised in one reaction do not contaminate another is to covalently cross-link the two strands of the newly synthesised PCR products by exposing them to ultraviolet (UV) light (12). Routine UV treatment of reactions immediately after amplification will prevent them from acting as templates in subsequent PCRs but may also prevent cleavage by some restriction enzymes. We have found that UV pre-treatment of pre-PCR reactions prior to addition of target DNA and the thermostable polymerase, prevents contamination which may occur in PCR reagents. Another similar approach is to add DNAse, an enzyme which rapidly degrades double stranded DNA, then heat the reaction to 98°C to denature the enzyme before adding target DNA and thermostable DNA polymerase. Because these approaches involve addition of reagents *after* the sterilisation step, they do not eliminate contaminating amplified fragments which may occur in the pipette barrels, the template DNA, DNA extraction reagents or in the thermostable polymerase itself.

Perhaps the most conclusive way to date of avoiding all contamination is to use deoxyuridine triphosphate (dUTP) in addition to the deoxynucleotides in all PCR reactions. dUTP is an analog of dTTP and is incorporated into all amplified fragments by *Taq* polymerase. DNA containing dUTP can then be selectively degraded by the enzyme uracil-N-glycosylase (UNG) (13) prior to each subsequent PCR. Use of dUTP in PCR does not affect yield, and amplification products can still be manipulated by restriction enzymes. Since UNG will not affect normal DNA, unincorporated dUTP or deoxyuridine in RNA, pre-PCR mixes which include *all* reagents can be pretreated with this enzyme, followed by heat denaturation and normal cycling.

Detection of PCR products

The simplest way to detect PCR products is by electrophoresis in a polyacrylamide or agarose gel, followed by staining with the dye ethidium bromide which fluoresces over UV light. Amplified bands of the correct size can be visualised over a UV transilluminator and photographed. In most instances identification of a band of the correct size is sufficient for diagnosis since a well designed PCR with a high annealing temperature will be so specific that the need for confirmation is abrograted. In some instances however it is necessary to confirm the identity of the amplified band. One example is in the amplification of HCV RNA where more than one PCR product occasionally results. The ultimate confirmation of the identity of an amplified DNA fragment is to determine its sequence. DNA sequencing though requires specialised equipment, is time consuming, expensive and not suited to diagnostic applications. There are, however, a number of ways of determining the identity of an amplified fragment without sequencing and which are amenable to the diagnostic setting.

Probing is an effective way of determining the identity of a DNA fragment of interest. A probe, like a primer, is a piece of single stranded DNA which is complementary to the DNA of interest. Probes need to be labelled to enable visualisation of their hybridisation, or lack of it, to a target sequence. To confirm the identity of the amplified HCV band we routinely probe the amplified product with a single stranded oligonucleotide 30 nucleotides in length. This oligonucleotide is complementary to a known HCV sequence which occurs within the amplified product.

Restriction enzyme analysis, like probing, allows identification of an amplified DNA fragment by confirming part of its known internal sequence.

Nested PCR is another approach to identification of an amplified band. It involves use of primers internal to the amplified band to do a second PCR using the first PCR products as a template (ie: doing a PCR of a PCR). Because nested approaches involve use of PCR products as templates, they necessitate the presence of PCR products in the prePCR area and so are a potent cause for contamination. We consider that nested PCR should be avoided in the diagnostic laboratory because of the contamination problems it can cause.

Conclusion

While PCR is a useful and relatively inexpensive technique with vast application to the clinical diagnostic laboratory, its introduction to this setting must be carefully planned in order to avoid potentially expensive contamination problems. The potential for contamination in the diagnostic laboratory is compounded by the fact that large numbers of samples are amplified using the same set of primers. For this reason contamination with previously amplified PCR products is potentially a considerable problem in the diagnostic laboratory. Meticulous application of the anti-contamination measures described in this article together with the use of dUTP and UNG in PCR set-up should avoid contamination. For trouble free diagnostic PCR it is essential that staff are well trained and familiar with the contamination potential of PCR.

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MARCH 1993

NZIMLS CONTINUING EDUCATION

SPECIAL INTEREST GROUPS

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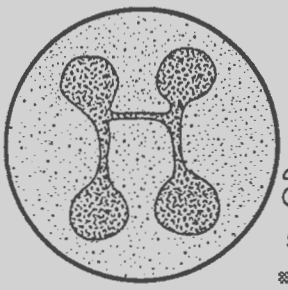


haematology

SPECIAL INTEREST GROUP

Convenor: Rennie Dix
Contact Address: C/- Anne Cooke, Laboratory Training Centre, Building 18, Auckland Hospital, Park Rd, Auckland. Fax (09) 307-4939

With the degree courses at Massey and Otago underway and the possibility of a third degree proposed by the Auckland Institute of Technology, the profession needs to consider the level of practical or clinical experience required by these students, before being eligible to apply for registration.

Each degree course needs to be evaluated with consideration given to the level of practical experience attained prior to and post graduation, and this measured against the MLTB's proposed competency document.

The NZIMLS Council has asked the MLTB to consider that degree students should have a period of not less than 6 months practical experience prior to application for registration as medical technologists.

Our concern is that less than one year's practical experience, especially bearing in mind the MLTB's requirement for qualification in two disciplines, will not provide a technologist with the same level of practical ability or clinical experience as the current technologist and will result in a downgrading of the profession's present standards.

The Haematology discipline, along with other SIG's, recommends that one year post degree in a discipline is a minimum requirement and invites comment from other members on this matter.

Haematology Seminar

A one day Seminar will be held on Saturday, 29th May, 1993

in the
Ernest & Marion Davis Post-Graduate Medical Centre,
Auckland Hospital.

Blood Films Revisited

An interactive session on blood film interpretation and the use of parameters in the diagnosis of disease.

IMMUNOLOGY

SPECIAL INTEREST GROUP

Convenor: Gillian McLeay
Contact address: Laboratory Training Centre, Building 18, Auckland Hospital, Private Bag 92024, Auckland.

NDMLS CLASS OF '89: FIRST STUDENTS GRADUATE.

Four years of study have finally come to an end. Official graduation day was 1 February, 1993.

A number of Auckland immunologists have taught the Immunology classes from time to time over the last two years, but it would be fitting to pay tribute to the two official Immunology tutors for the class of '89.

Tricia Lucas from the Department of Applied Science at the Auckland Institute of Technology (AIT) introduced the students to Immunology in the second year, and John McKay took over from there.

John was a hard taskmaster, whose high standards were hard to meet, but the students appreciated the discipline he imposed and the effort he put in; with such encouragement, they rose to the occasion magnificently.

On behalf of the Immunology Special Interest Group, I should like to honour those graduates from the class of '89 who took Immunology as one of their "Diploma level" subjects. ("Diploma level" is equivalent to "Certificate level" in the MLTB Diploma of Medical Laboratory or Technology qualification).

These graduates form an interesting and diverse group. I should like to introduce them to ISIG and NZIMLS members with the following *"pen portraits"*.

Tracy Bathgate

Tracy is the daughter of Perry Bathgate who trained as a Medical Laboratory Technologist, and now holds a very senior position with Salmond Smith Biolab in Auckland. Tracy worked off and on at her Dad's company from the end of 1984 to 1988 - as a result she loves rabbits!

Educated at Auckland Diocesan School for Girls, Tracy had a successful school career, gaining the Australian Mathematics Certificate 1983 to 1987 inclusively.

In sport, she gained her school colours for trampolining. She was School Trampoline Champion and School Diving Champion. She also played for the Second Eleven and once reached 96 not out!

On the cultural side, Tracy played in the school orchestra and sang in the choir.

In the NDMLS course, Tracy majored in Immunology (she was the first to sit a Major level examination in Immunology), with Microbiology as her Minor and Haematology as her Elective subjects. She is very proud of the Grade A she got for Microbiology.

Her great achievement outside the Laboratory is trampolining, with 1 gold and 3 silver world championship medals so far, and another silver on the way. She has been a NZ team representative since 1983, and was NZ Team Captain when the team went to USA in 1988.

In the midst of all this she finds time for workouts at the gym. aerobics, running for health and learning to speak French.

Tracy has chosen to work in Microbiology at the Auckland Children's Hospital, and plans to upgrade her Microbiology qualification to a Major level this year.

She has been selected to represent New Zealand at the World Games — the "Olympics" for the sports that are not represented in the Summer Olympics, and will be going to the USA at the end of July.

Future plans? Tracy enjoys a challenge and succeeding by her own efforts — ultimately she would like to be involved in research.

Susan Baxter

Sue is one of the successful NDMLS graduates from the private sector, completing most of her training at Medlab in Auckland, apart from four weeks in the latter part of her fourth year, when she joined the Immunology Department at Auckland Hospital to complete some sections of her log book.

Sue attended Westlake Girls High School on Auckland's North Shore. She is a typical Technologist with proven academic and practical skills, and a range of outside interests, especially in sport.

Modest about her achievements, it was hard to get Sue to acknowledge being the success she undoubtedly is. She majored in Immunology, took Clinical Biochemistry as her Minor level and Cytogenetics as her Elective subject. She loved cytogenetics (due in no small way to Alice Benson, the tutor), but unfortunately there are not too many trainee positions or employment opportunities in that discipline.

The highlight of the four year NDMLS course for Sue was coming top in Immunology — a splendid effort.

Her interests include water sports, horse riding and tennis — she did admit to winning trophies in all of these, but would not specify what they were.

Sue has just commenced employment as a Staff Technologist at Medlab in Auckland. She is attached to the Immunology Department, but can often be found working in Biochemistry, and on the day I rang her she was in Microbiology — a truly versatile lady.

Sue is having a break from study this year, but future goals include upgrading her qualification to degree status, travel and research work overseas.

Sharon Clode

Sharon's looks are deceptive; very slim and slight of build, she looks as though a strong puff of wind would blow her over. It is an illusion. Sharon is one of New Zealand's leading track athletes.

It all began at Takapuna Grammar, another North Shore school in Auckland, which has a fine record for sporting as well as academic excellence. There Sharon began her sporting career, winning the Sports Award for Cross Country and Track and gaining an Athletics Blue.

In addition, she demonstrated a talent for sewing and won the school's clothing award. Sharon designs and makes all her own clothes, and does for her friends also, time permitting.

In the NDMLS course Sharon majored in Immunology, with Microbiology as her Minor subject and Haematology as her Elective.

During Term 2 of 1990 she had to get special leave of absence for 7 weeks from AIT to travel to Japan and Belgium to represent NZ in the IAAF Cross Country championships. A great honour, but she also had to complete all her course work and pass the module. It was not easy, but she did it.

The highlight of the NDMLS course for Sharon was simply completing it successfully.

Other achievements include taking part and doing well in the 1991 NZ Cross Country championships, and representing NZ for three consecutive years (1991, 1992 and 1993).

Her interest in sewing has led to her designing and making sportswear and bridal garments.

Sharon's great love is cats — and one in particular. Abdile, her tabby Abyssinian age 3, is named after a famous runner from Somalia, the current World 1500 Metres Champion. He is visiting NZ this summer and is going to have his photo taken with his "namesake". Abdile's (the cat) photograph has pride of place on Sharon's workbench.

1993, despite being free of study commitments, is going to be another busy year for Sharon and her husband and fellow athlete, Phil Clode.

Phil is going to the World Indoor Championships (running) at Toronto in March, while Sharon is going to Spain the same month for the World Cross Country Championships in Spain. There is a possibility she will be going to Sweden in May also.

For the immediate future Sharon intends to work as a Staff Technologist in the Virology/Immunology Department at Auckland Hospital, continue her running career and carry on with sportswear production.

It sounds an impossible timetable, but Sharon has opted for a part-time position, and with her dedication and determination, she should be able to achieve all her dreams and ambitions.

Angela Gardiner

Angela trained at the Diagnostic Laboratory which is the other Community Laboratory in Auckland. This laboratory, like its rival Medlab, has had a strong tradition of training over the years.

Angela attended Northcote College — the North Shore appears to be a rich source of potential Technologists!

On the subject of achievements, other than gaining her National Diploma in Medical Laboratory Science. Angela maintains she is "not very exiciting" and "put everything on hold for four years" to concentrate on her studies for the NDMLS course. "It was good to pass — a bit of a struggle. — but wortwhile."

Angela majored in Haematology, with Immunology as her Minor and Cytogenetics as her Elective subject choices. She would have liked to major in Immunology, but this was not possible.

Currently working at Diagnostic Laboratory as a Staff Technologist in Haematology, she is content, for the time being, to have the opportunity for relaxing and sleeping — sadly lacking in the last four years.

As for the future, she views it as a time for building on existing knowledge, improving practical skills and just keeping on learning....!

Lynley Henderson

Lynley laughs at being considered one of the NDMLS course's "mature students"; she uses a slightly more derogatory term — "granny brigade". However, this is a complete misnomer for a vital and energetic lady who was only a little older in years than the average age of the class. Her enthusiasm and outlook put her right on a par with her classmates, while her maturity was an added advantage in supplying the right attitude and discipline towards her study.

Lynley, originally a Mainlander until she defected to the North, says she owes a lot to the excellent teaching programme at Gore High School where she was a student.

She and her school were very proud when she was selected as an American Field Scholar in her 7th form year. Lynley regrets not finishing the 7th form, but says she gained a lot academically from her time in the USA, and was awarded

an Athletics Blue into the bargain.

Immunology was always Lynley's greatest love, so there was no question about what her choice for her Major subject would be. Her Minor subject was Clinical Biochemistry, which she thoroughly enjoyed and found complemented and enhanced her Immunology. Haematology as her Elective rounded off a very satisfactory educational programme.

Academic success was rewarded by winning the Hyde Instruments prize for Chemistry at AIT, and the Laboratory Service's prize for top student overall for Years 3 and 4 of the NDMLS.

On other achievements Lynley highlights becoming proficient at word processing which was essential to keep up with John McKay's weekly Immunology assignment programme. He required no less than a standard suitable for publication for each one!

Favourite pastimes are embroidery and reading, but there has been not much time for that in the past four years.

However Lynley's great hobby is singing, and in particular she sings with a Barber Shop Quartet which she says is fun and a great way to meet people. The choir came second at the National Championships.

Plans for 1993 — ".....Work". Lynley has been appointed to a Staff Technologist position at Virology/Immunology at Auckland Hospital. She has no definite plans yet for the future, except that it will involve some type of study.

Sarah Perry

Sarah's vibrant personality is mirrored by her hair — the colour of beech leaves in Autumn. Quietly spoken, but positive and outgoing at the same time, she sets very high standards for herself, and expects similar commitment from others. She has some firm ideas on training, which should be interesting for the trainees who come in contact with her later this year.

Educated at Massey High School in the western suburbs of Auckland, Sarah, on leaving school worked for a year as a Laboratory Assistant in the Clinical Chemistry Department at Auckland Hospital. During this time she successfully completed the first year of the QTA course which in Auckland is run by AIT.

Sarah chose Clinical Biochemistry as her Major subject, with Immunology as her Minor and Haematology as her Elective. Her four years in the NDMLS course had their moments, but she acquitted herself very well, and the difficulties she encountered only served to enhance her personal growth and maturity. It is these attributes, in addition to her practical skills, which are valued by her colleagues and departmental heads.

This year Sarah will find herself standing on the podium at one or two prizegiving and graduation ceremonies, accepting acknowledgement of her commitment and achievements.

Sarah's outside interests reflect her healthy lifestyle, and include touch rugby, aerobics and weight training. She is modest about achievements in these sports, (especially touch rugby) and tends to dismiss them lightly.

1993 brings the opportunity for a short (but long overdue) holiday overseas. However, Sarah also has her eyes set on upgrading her Immunology qualification. She successfully applied for a Staff Technologist position in the Virology/Immunology Department at Auckland Hospital.

As for the future, she intends to extend her skills. In her present position she will have the opportunity to work in the Virology section as well.

At some time she would like to return to Biochemistry, but she has a few other goals to achieve first.

Course Announcement

Introduction to Molecular Genetics
and Gene Manipulation

A one week non-credit introductory workshop will again be conducted in the Microbiology and Genetics Department of Massey University during the May Holidays 1993, namely 10-14 May. The aim of the course will be to provide for those people who may have a potential professional interest in the subject, a working introduction to the powers and limitations of the techniques. This year we shall endeavour to focus on topics in applied molecular genetics relevant to agriculture and medicine. Lecture material to be covered will include DNA and genome structure, the molecular genetics of plasmids and transposons, basic strategy of recombinant DNA research (both basic and applied) and the quasi-legal aspects of "genetic engineering". Practical work will include plasmid isolation, transformation/electroporation, restriction enzyme mapping, DNA cloning, PCR and RFLP analysis. Background assumed will be the equivalent of Introductory Genetics and Introductory Biochemistry (200-level).

Although Boehringer-Mannheim are continuing their generous sponsorship for this course in the form of biological materials, there will be a charge of $400 (plus GST), in order to cover the cost of additional materials and facilities. Accommodation will have to be arranged off campus, as unfortunately, extramural fully books the campus accommodations. The enrolment will be limited to 24 (the capacity of the teaching laboratory). For further information and an enrolment form, please contact: Associate Professor Eric Terzaghi (Extn 8704) or Dr Rosie Bradshaw (Extn 8219), Department of Microbiology and Genetics, School of Biological Sciences.

EARLY NOTICE

AACB New Zealand Branch Education Meeting 1993 Hamilton

The second annual AACB Branch Education Meeting will be held in Hamilton around mid year. The meeting will be once again aimed at examination candidates preparing for examinations in Clinical Biochemistry at all levels from Laboratory Assistant upwards. There will also be plenty to be learnt by those who wish to have an update on current laboratory and clinical practise.

For further information contact:

Don Mikkelsen
C/- Biochemistry
Waikato Hospital
Pvte Bag 3200
Hamilton
Phone: 07 839 8616
Fax: 07 839 8759

NEW ZEALAND INSTITUTE OF

MEDICAL LABORATORY SCIENCE

EXAMINATION LIFTOUT

Specialist Certificate Regulations
Specialist Certificate Examination Application Form
Q.T.A. Regulations
Q.T.A. Examination Application Form
N.Z.I.M.L.S. Membership Application Form

The New Zealand Institute of Medical Laboratory Science offers to medical laboratory assistants the qualification known as the Certificate of Qualified Technical Assistant (QTA) and to medical laboratory technologists the qualification known as the Specialist Certificate.

The Examinations Committee is based in Christchurch and all correspondence should be addressed to:—

The Executive Assistant
N.Z.I.M.L.S.
P.O. Box 3270
Christchurch
Phone/Fax (03) 313-4761

NEW ZEALAND INSTITUTE OF MEDICAL LABORATORY SCIENCE SPECIALIST CERTIFICATE EXAMINATION

EXAMINATION SUBJECTS

The examination is offered in:

Clinical Biochemistry
Haematology
Histology
Nuclear Medicine
Cytogenetics
Microbiology
Immunohaematology (Transfusion Science)
Medical Cytology
Immunology
Virology

PREREQUISITES

1. Candidates for the examination must have passed a Certificate Examination offered by the Medical Laboratory Technologists' Board or be granted an exemption by the Council of the NZIMLS.

2. **Candidates must be financial members of the NZIMLS at the time of sitting the examination and be a financial member or have submitted a valid membership application form at the time of applying to sit the examination.**

SYLLABUS

Copies of the syllabus are available from the Executive Officer of the NZIMLS, P.O. Box 3270, Christchurch.

EXAMINATIONS

1. The examinations will be held annually during November.

2. Candidates must complete the application form and forward this, complete with examination fees, to the Executive Officer of the Institute before the closing date. **No late applications will be accepted.**

3. Candidates must be financial members of the NZIMLS at the time of sitting the examination.

4. The examination consists of two written papers each of three hours duration.

5. To pass the examination candidates must obtain an overall mark of 50%.

6. The results of the examinations will be announced by the New Zealand Institute of Medical Laboratory Science. Successful candidates will be awarded the NZIMLS Specialist Certificate in the appropriate discipline.

7. The candidate's script will be returned upon receipt of a written request by the candidate. No copy will be retained and no correspondence relating to the marking of the script will be entered into.

NEW ZEALAND INSTITUTE OF MEDICAL LABORATORY SCIENCE

Application to sit Specialist Certificate Examination 10th and 11th November 1993

SECTION A — TO BE COMPLETED BY THE CANDIDATE

Name: Mr / Mrs / Miss .....
(Surname) (First Names)

Laboratory .....

Laboratory Address .....

.....

Examination Subject .....

Medical Laboratory Technologist Board Certificate Examinations passed:

Subject ..... Year Sat .....

Subject ..... Year Sat .....

EXAMINATION FEE: $400 (GST Inclusive)

The full examination fee must be paid with the application.

SECTION B — TO BE COMPLETED BY THE PRINCIPAL OR CHARGE TECHNOLOGIST

"I certify that the above candidate will meet the requirements of the Specialist Certificate Examination"

Signed .....

Designation .....

Please state the name and address of the person responsible for receiving the papers and supervising the Examination in your laboratory or centre.

Name .....

Address .....

.....

.....

APPLICATIONS CLOSE FRIDAY 28 MAY, 1993

Please forward application forms accompanied by fees to: Executive Officer, NZIMLS, PO Box 3270, Christchurch.

NO LATE APPLICATIONS WILL BE ACCEPTED

Special Note to Applicants

*If not already members of the NZIMLS applicants to sit this examination **must** submit a valid membership application along with this examination application.*

NEW ZEALAND INSTITUTE OF MEDICAL LABORATORY SCIENCE CERTIFICATE OF QUALIFIED TECHNICAL ASSISTANT

EXAMINATION SUBJECTS

Public Health Microbiology
Clinical Biochemistry
Cytogenetics
General Certificate (See prerequisite 2)
Haematology
Histological Technique
Medical Cytology
Transfusion Science (Blood Products)
Medical Microbiology
Mortuary Hygiene and Technique
Radioisotopes and Radioassay Technique
Transfusion Science
Immunology (Microbiology)
Immunology (Tissue Typing)

PREREQUISITES

1. Candidates for the examination must be employed as medical laboratory assistants in an approved laboratory and have worked continuously in the subject for two years prior to the examination or accumulated not less than two years practical experience in the examination subject.
2. Small laboratories which require their medical laboratory assistants to work in more than one subject can apply to the NZIMLS for students to train for the General Certificate Examination.
3. Candidates for the Immunohaematology Examination must have completed not less than 320 hours and candidates for the General Certificate Examination not less than 160 hours in practical cross-matching of blood for clinical use.
4. **Candidates must be financial members of the NZIMLS at the time of sitting the examination and be a financial member or have submitted a valid membership application form at the time of applying to sit the examination.**

SYLLABUS

The syllabuses for all subjects are available from the NZIMLS, P.O. Box 3270, Christchurch.

(NOTE: LATE APPLICATIONS WILL NOT BE ACCEPTED)

EXAMINATIONS

1. Q.T.A. candidates who began their practical experience, on or before 31 January, two years prior, will be eligible to sit the examination.
2. Candidates must complete an examination application form and forward this, together with the appropriate fee, to the Executive Officer before the closing date.
3. The examination will consist of two written papers, each of two hours duration. Candidates for the Medical Cytology Examination will also be required to complete a practical examination.
4. The candidate must obtain an overall mark of 50% to pass the examination. Candidates for the General Certificate Examination must obtain a minimum of 40% in each of the four sections and 50% overall to pass the examination.
5. The results of the examinations will be announced by the New Zealand Institute of Medical Laboratory Science.
6. The candidate's script will be returned upon receipt of written application by the candidate. No copy will be retained and no correspondence relating to the marking of the script will be entered into.
7. Candidates must be financial members of the NZIMLS at the time of sitting the examination.

NEW ZEALAND INSTITUTE OF MEDICAL LABORATORY SCIENCE

Application to sit the Examination of Qualified Technical Assistant 2nd and 3rd November 1993

SECTION 1 — TO BE COMPLETED BY THE CANDIDATE

Name: Mr / Mrs / Miss ..... (Surname) (First Names)

Laboratory .....

Laboratory Address .....

.....

Subject (Haematology, Microbiology, etc) .....

EXAMINATION FEE: $80 (GST Inclusive)

The full examination fee must be paid with the application.

SECTION B — TO BE COMPLETED BY THE PATHOLOGIST OR CHARGE TECHNOLOGIST

Date candidate commenced work in examination subject .....

"I certify that the above candidate meets the requirements of the Q.T.A. Regulations"

Signed .....

Designation .....

Please state the name and address of the person responsible for receiving the papers and supervising the Examination in your laboratory or centre.

Name .....

Address .....

.....

.....

Office use only

APPLICATIONS CLOSE THURSDAY 28 MAY, 1993

Please forward application forms accompanied by fees to: Executive Officer, NZIMLS, PO Box 3270, Christchurch.

NO LATE APPLICATIONS WILL BE ACCEPTED

Special Note to Applicants

*If not already members of the NZIMLS applicants to sit this examination **must** submit a valid membership application along with this examination application.*

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

Application for Membership (For use with Examinations only).

(Please Print Clearly and Tick Appropriate Box)

I, _____

SURNAME _____

MR, MRS, MS, MISS _____

INITIAL(S) _____

FIRST NAME(S) _____

OF,

WORK ADDRESS _____

_____

Hereby apply for membership of the New Zealand Institute of Medical Laboratory Science in the category of:

☐ Member ☐ Associate

AND Certify That I Have:

☐ Not Previously Been a Member ☐ Previously Been a Member (State Category: ___)

☐ Resigned (Date:) _____ ☐ Did Not Resign

I am employed as: _____

in the Speciality Department of: _____

Highest Professional Qualification: _____ Year Obtained: _____

Nominated By: _____

(Current Financial Member N.Z.I.M.L.S.)

Please forward payment with Application for Membership, to the Executive Officer, NZIMLS, P.O. Box 3270, Christchurch.

Current Membership Subscriptions are:

MEMBER $88.40 (GST incl.) ASSOCIATE $33.80 (GST incl.)

Member — any person who is registered by the Medical Laboratory Technologists Board

Associate — any person engaged in Medical Laboratory Science who is not eligible for any other class of membership.

The appropriate membership subscription must accompany this application for this to be a valid application.

TRANSFUSION SCIENCE

SPECIAL INTEREST GROUP

Convenor: David Wilson

Contact Address: Manawatu Regional Blood Centre, Palmerston North Hospital, Private Bag, Palmerston North.

AUDIO UPDATES

These cassette tapes, with written transcripts, are available through the TSSIG and are a convenient way to use your spare time for continuing education — perfect for making traffic jams tolerable, or for sharing with your colleagues at lunch time. There are now nine topics available. See the advertisement elsewhere in this newsletter.

The 08/92 issue of Audio Updates is entitled: 'Approaches to Bloodless Surgery'.

The topic is discussed by Dr Richard Spence, the Head of General Vascular Surgery at Cooper University medical Centre in Camden, New Jersey, USA. Dr Spence's research has involved Fluosol, Jehovah Witnesses and ways to minimise blood loss during surgery.

Dr Spence talks about pre-operative, intra-operative and post-operative measures to reduce surgery-related allogeneic blood transfusion. Pre-operative techniques include autologous predonation of blood and efforts to increase red cell mass prior to surgery such as the use of erythropoetin. The intra-operative measures discussed are primarily under the control of the surgeon and perfusionist. Post-operative nutritional support, avoidance of unnecessary phlebotomy and reasoned clinical assessment of each patient before transfusion are all valuable in limiting exposure of patients to allogeneic blood transfusion.

BOOK REVIEW

Case Studies in Transfusion Medicine

by Hoffstadter, De Christopher, Perkins and Berte, published in 1992 by the American Society of Clinical Pathologists.

This is a series of case studies with questions for consideration to guide the reader through each problem to its solution. Answers and discussion about each case are in the second part of the book.

There are sections on donor evaluation, grouping discrepancies, antibody identification, transfusion therapy, therapeutic apheresis, adverse effects of transfusion, haemolytic disease of the newborn, and parentage testing. Each section is introduced with a suggested reading list to orient the student to the topic.

This book is designed to emphasise problem-solving skills rather than mere recall or interpretation. In spite of its significant clinical content and predictable American slant, it could be a valuable educational tool for senior trainees, registered transfusion science technologists, and immunohaematology registrars.

ARTICLES OF INTEREST

Report from the N.Z. Blood Transfusion Advisory Committee meetings, 9-10th July, 17-18th December 1992. Compiled by Dr Steve Gibbons

Donor Registration Form

The health screening questions were reviewed and a new version of the form was printed after the July meeting. In December it was decided to add a new question 'Have you been overseas?' which will be added at the next re-print.

Donor selection

Donors providing platelets to a random donor pool must have taken no non-steroidal anti-inflammatory drugs (NSAID's) for at least 24 hours and no Aspirin for at least 3 days.

People with Gilbert's disease are acceptable as blood donors.

The medication list used by the NZBT Services is to be revised. The NZBTAC may adopt, with modifications, the list generated by the Australian Red Cross BTS which is somewhat more concise than the New Zealand version.

Section 3 of the NZ Standard Operating Procedures draft was modified to make it mandatory for 'all donors' to be interviewed with particular attention to new donors.

Blood Bag Promotion Label

Otago is to print a promotional blood bag label which states 'a gift of life freely donated by a caring kiwi'. It may be possible to obtain a small number of these from the Otago BTS.

Advertising Funding

There were concerns expressed regarding the inadequacy of some advertising budgets. Transfusion Services are encouraged to write this activity into their budgets.

Hepatitis C

In July it was announced that $1.642 million was to be allocated for Hepatitis C screening. This was to include funding for the development of educational materials, confirmatory testing, purchase of test kits and for staff resources. Confirmatory testing was ultimately to be performed by the New Zealand Centre for Disease Control.

It is proposed to collect data concerning test kit performance similar to that which the NZBTAC collects for HIV test skits.

Donors who are HCV-RIBA indeterminate are to be deferred from donating.

HCV antibody (+), PCR (-), RIBA (-) are to be deferred and retested after one year.

Look back programmes

Recent clinical events in which recipients of HCV contaminated blood have died some years after the transfusion of complications related to Hepatitis C were discussed. It was proposed Blood Transfusion Services should test recipients of locally produced blood and blood products from repeatedly anti-HCV reactive blood donors. The logistical and financial implications are likely to be considerable and the issue is to be discussed again at the next BTAC meeting.

Zoster immune globulin

This product is in short supply and more hyper immune plasma is required. Suitable donors are patients who are recovering from Shingles and in whom the skin lesions are dry. The minimal acceptable titre of antibody is 128.

Intravenous tetanus immunoglobulin

Current usage is 12 vials per annum. It is suggested that each regional centre hold at least one vial.

Anti-D in Intragam

To minimise, a recurrence of an unacceptable level of anti-D present in Intragam, plasma with an anti-D titre of 16 or less should not be sent for immunoglobulin.

The safety of this CSL product regarding HCV transmission was discussed. Historically this product has not been associated with the development of nonA nonB hepatitis. The BTAC endorsed the safety of this product in respect of HCV transmission.

NZBTS Standards

In July the BTAC met with representatives of the Therapeutic section of the Department of Health to discuss the development of NZBTS Standards and Accreditation of Blood Transfusion Services. The Australian BTS Standards, which are written to ISO 9,000 Standards, were tabled. After some discussion it was proposed, in the interim, to adopt the Australian Standards. A working party was constituted to harmonise these Standards for NZ requirements. This document is hereafter called the Code. It was anticipated the NZ draft Standard Operating Procedures would be a subservient document as they are written in a more prescriptive mode and would serve as a useful guideline for the production of local Standard Operating Procedures.

The Code is to be completed by mid-March 1993. It is proposed to have, at the end of April, a workshop to discuss the Code, the development of SOP's and the audit process. The latter is to be conducted by the Therapeutic section of the Department of Health during May/June 1993.

The NZ Department of Health is to ask if NZ may be represented at future reviews of the Australian Blood Transfusion Service Standards.

Bone Marrow Donor Registry

From September 1991 to June 1992, the Australian BM Donor Registry has made 16 donor searches for New Zealand patients. Five potential bone marrow donors were identified. Of the 4 Maori or part Maori patients only one potential donor was found. A position paper from the NZBTAC concerning the development of a NZ matched unrelated bone marrow donor registry is to be submitted to the Department of Health.

Adverse effects of blood donation

A very small number of donors may experience a significant adverse effect following blood donations. It is recommended that donors not be charged for medical care arising from such adverse effects. Each Blood Transfusion Service needs to develop a policy, in conjunction with hospital administrators, to cover this situation. An abstract concerning the frequency and nature of some adverse events was published in Transfusion, 32; Suppl, A35, 875, October 1992.

ABO Incompatible Transfusions

These are still occurring from time to time. The BTAC wishes to capture more detailed information concerning these events. They want to determine how and why these events occur, what action was taken and what were the outcomes. A proforma is to be developed to capture this information.

HTLV1 Testing

The Australian Red Cross Blood Transfusion Service has recommended that all donations be tested for this infection. The necessity for HTLV1 testing in NZ was discussed. To date no confirmed cases of HTLV1 have been identified in targeted NZ blood donors, that is, those donors who were born in, or resident for one month or longer in an endemic area. It was proposed that an option paper be written concerning this subject.

Future BTAC meetings

As a result of recommendations from the Hepatitis C enquiry the BTAC membership is to be expanded to include a representative from the NZ Haematology Society, a doctor who treatas Haemophiliacs and a consumer.

A review of platelet serology

Alan Knight, Transfusion Medicine, Dunedin Hospital.

A number of conditions may adversely affect the response to platelet transfusions. These conditions may be either non-immune or immune in origin.

Those of non-immune origin include:
Splenomegally
Fever
Disseminated Intravascular Coagulation
Quality of the infused platelets

Those of non-immune origin include:
Alloimmunisation
Autoantibodies
Drug related antibodies
Circulating Immune complexes

The development of autoantibodies may be related to malignancy, sepsis or post bone marrow transplant.

Alloimmunisation to platelets may include HLA antibodies that are cross-reacting with platelets or platelet specific antibodies which may lead to neonatal alloimmune thrombocytopaenia (NAITP), post transfusion purpura (PTP) or platelet refractoriness following transfusion.

Neonatal Alloimmune Thrombocytopaenia (NAITP). The severity of this condition may range from fairly mild affecting few of the foetal platelets to its severest form resulting in spontaneous inercranial haemorrhage in the foetus. The mechanism of its development is similar to the familiar haemolytic disease of the newborn (HDN) due to red cell antigen incompatibility. However one major difference is that in 50% of reported cases of NAITP, the disease occurred during the first pregnancy.

Post Transfusion Purpura (PTP). The mechanism for this condition is of similar aetiology to that of red cell haemolytic anaemia due to immunisation by the red cell antigens and subsequent development of immune antibodies. However unlike red cell mediated haemolytic anaemia, in PTP the platelet antibodies not only sensitize the donor platelets but also affect the patient's own platelets resulting in a total depletion of circulatory platelets so that any platelets, even those of identical type to those of the patient, may be sensitized and thus no increment in the platelet count following transfusion is observed.

Platelet Refractoriness. This is a similar condition to PTP but in this condition only platelets carrying the antigen to which the patient has been sensitized will be affected and thus platelets compatible with the patient will remain viable.

Platelet Specific Antigens. A number of platelet specific antigens have been identified. Over time, various workers assigned different specificities but subsequently a number have been shown to be identical and more standardisation has occurred.

Antigen	*Frequency*	*Previous Identity*
Pla^1	97-98%	Zw^a
Pla^2	26%	Zw^b
Ko^a	12-17%	
Ko^b	99%	
Bak^a	85-90%	
Bak^b	62-70%	
Pen^b	1%	Yuk^a
Pen^a	99%	Yuk^b
Br^a	21%	
Br^b	99%	
PI^{E_1}	99.9%	
PI^{E_2}	5.0%	
Sib^a	25%	$?Ko^a$
Nak^a	96%	
Duzo		

Anti-Pla^1 is the most common cause of NAITP and PTP.
Anti-Pla^2, anti-Bak^a and anti-Br^b are the antibodies most commonly associated with platelet refractoriness following transfusion.
Anti Duzo- only one example has ever been found in 30 years since it was first described and the donor has subsequently died and no further serum is available.
Most reported cases of anti-Ko^a and anti-Ko^b have surprisingly been of the immunoglobulin class IgM.

There does seem to be some variation in frequencies in different racial groupings.

Techniques for the Detection of Platelet Specific Antibodies

A variety of techniques have been developed which in the main are based upon standard red cell serological techniques but modified to suit when used with platelets. These include direct agglutination, when IgM antibodies are suspected, to modified indirect Coombs technique using fluorescein conjugated anti-human globulin serum with the resulting reactions examined by fluroescent microscopy. EIA techniques employing microplates coated with immobilised platelets, have also been developed.

If autolantibodies are suspected a modified direct Coombs test can be employed using fluorescein conjugated anti-human serum.

Blood Donor Recruitment

Grant Storey, Waikato Regional Blood Centre, Hamilton

Altruism remains the most often cited reason for donating blood, although needs for self-esteem continue to play a role. The donor's blood is seen as a gift, as an act of generosity towards a person or persons less well off. It represents a co-operative act of commitment to the community at large, and while the donation remains anonymous, it never the less establishes the donor's personal relationship to their fellows within the community. No clear picture of the personality of potential blood donors emerges from research, but what is clear is that the blood supply is heavily dependent upon a core of committed regular donors.

Research continues to indicate that the pressure to conform to the expectations of others may be a powerful factor motivating blood donation. Face to face solicitation has been reported to be a highly effective recruitment rechnique. A personal reminder call the night before a pledged donation has been found to have quite an effect on donors keeping their appointments. In one report, investigators stated that a primary reason given for non donation among respondents to a survey was that they had never been asked. In fact non donors gave their highest ratings to the statement, "Nobody asked me personally". Mass appeals did not count as their actually having been asked.

The association of fear with avoidance of donation has been well documented. These fears include fear of the needle, pain, sight of blood, weakness and dizziness. Some people also have the fear of contracting AIDS by donating blood. The length of time it takes to donate is cited by donors and non donors alike as a contraint against donation. In recent studies, the reason for non donation most often mentioned was a medical problem. As noted by one of the researchers, the real question is whether these are actual beliefs of the non donors or whether they are really excuses and rationalizations for not donating. Being deferred from donations is, or can be, a psychologically distressing experience and the effect on the return rate of temporary deferred donors is stronger in early career donors. The focus of donor recruitment staff needs to be on the retention of donors, specifically on those at low risk for infection with transfusion transmitted diseases. Second time donors should be recalled as soon as they become eligible for their next donation. The first and second donations themselves are critical; deferral, reactions, pain and anxiety lead to lower return rates. Blood collection staff are central in making those

experiences as positive as possible. While the aging of society will result in an increased demand for blood components, this demographic trend also has the potential to provide a source of healthy donors. It has been said that there may be a positive psychosocial impact on older donors, "in which they can gain gratification and sense of fulfillment from performing this altruistic act at a time in life when such opportunities are being withdrawn". The advantage in terms of reduced risk from AIDS infection, by using such a population should be evident as well. Between the ages of 65 and 70, these reliable donors are likely to make more donations than most donors do in a life time. There is a study reported on this group of possible donors in Transfusion 1991: 31 (8) 693-697 "Characteristics of Elderly Blood Donors".

At the 1990 combined ISBT/AABB Joint Congress which I attended, the poster sessions contained many ideas for the recruitment and retention of blood donors. Unfortunately, however, most posters dealt with situations involving large communities and the use of full time professional donor recruitment staff which most of the New Zealand blood donor services do not have. The recent ISBT Congress held in Brazil did not offer any information to me on this subject. I have picked out information from a few of the 1990 abstracts which may be of local interest.

1) "Thank You for Donating"
"Thank You For Donating" calling programme was initiated. The goal was to increase donor retention by providing a dfferent type of customer service. The calling involved two steps:
a) Thanking the donor for taking the time to donate,
and
b) Reminding them of their eligibility date for their next donation.

These calls were genuinely accepted by the donors and an unexpected benefit from the programme developed in that some donors voluntarily set appointments for their next donation. These courtesy calls had a positive impact on donor attitudes.

2. Duffers and Donors
This was an idea of a golf tournament for blood donors. The original concept for the entry fee into the tournament was one blood donation. By securing the use of a prestigious golf club, donors were offered a chance to play on a course not normally available to the average golfer. This is now an annual event which has brought in many first time donors.

3) A Corporate Blood Donor Campaign
Key strategies in donor recruitment and motivation known to produce results are:
Promoting general awareness of the blood drive, direct donor solicitation, use of donor captains, publicity and recognition for captains and donors. The effects of the commitment of top management in a corporation to provide volunteer community service such as blood donations was found to be an effective campaign way of rapidly increasing blood donations.

As an aside I also noted in the Continuous Flow magazine a heading "Just FAX when you need a donor". One sentence from that article — "When people get a FAX message from the Red Cross, rest assured they know it's based on urgency and need and they respond".

Finally I would like to refer you to a review in Transfusion from which I obtained information for this article. It is entitled "Why do they give the gift of life?" J.A. Piliavin. Transfusion Volume 30 Number 5 1990.

Assessment of FVIII:C from random donors
Michelle de Koster, Les Milligan, Dr JM Faed, Otago Regional Blood Service, Dunedin.

Haemophilia A is a recessive genetic disorder of human coagulation factor VIII production due to a structural mutation at an X chromosome locus resulting in production of a factor VIII complex which lacks coagulant activity.

Treatment of patients suffering from Haemophilia A requires replacement of their congenitally deficient coagulation factor. The simplest treatment is fresh human plasma, however problems of circulatory overload and loss of factor VIII coagulant activity while storing plasma, mean factor VIII is more conveniently given as a concentrate.

The preparation of cold insoluble plasma protein (cryoprecipitate) solutions is a convenient method of obtaining concentrates of factor VIII and since its description in 1965 has become widely used in blood banks for factor VIII production.

These trials examined some aspects of the factor VIII manufacturing process as part of a feasability evaluation of a directed donor programme for supply of cryoprecipitate for selected Haemophilia A patients.

The trials assessed the yield of FVIII:C from plasmapheresis donors not boosted with DDAVP, the effect of small amounts of heparin in plasma during preparation of cryoprecipitate, and the effect of amicar (epsilon aminocaproic acid) and heparin during preparation of cryoprecipitate.

The addition of heparin at varying concentrations to plasma did not give rise to a significant increase in Factor VIII yield. Heparin, in association with amicar, gave an increase in overall Factor VIII yield. However the average percentage yield of 57.6% Factor VIII:C is not signficant when compared with an average yield of 68.2% Factor VIII:C obtained from DDAVP boosted donors.

Cadaver Kidney Donor Testing and Recipient Selection
John Dagger, Tissue Typing, Wellington Regional Blood Centre.

A. Potential Donor Testing
i) ABO Group.
An accurate ABO group is essential, both a cell and serum group are carried out, bearing in mind the possibility always of some centre transfusing group O blood to non-group O recipients.

ii) Infectious Disease Screening
Screening tests for HBsAg and anti-HIV must be negative. The anti-CMV status of the donor is also determined but does not influence recipient selection.

iii) Tissue Typing
The HLA Class I antigens (HLA-A locus and HLA-B locus) and the HLA Class II antigens (HLA-DR and HLA-DQ) are defined by the lymphocytotoxicity test.

iv) Direct Match
The equivalent of red cell compatibility testing. The latest serum samples of all individuals in New Zealand are reacted against the T lymphocytes of the donor to detect pre-existing HLA class I antibody, reactive with HLA class I antigens present on the cells of the donor.
Lymphocytes harvested from the peripheral blood are used for all tissue typing work locally, alternatives are lymphocytes harvested from the spleen or lymph nodes of the donor.

B. Recipient Selection
i) ABO Compatible
Same criteria applies as to red cell transfusions. Kidneys from group O donors are normally used for group O recipients only.

ii) Direct Match.
The Direct Match must be negative. This test is done in duplicate with extended complement incubation times. Failure to detect a positive result can result in acute rejection of the kidney.

iii) Tissue Typing
There are 21,697,200 different phenotypes possible of the

HLA-A, B and DR antigens. The larger the recipient pool the greater the chance of selecting a recipient that matches the HLA antigens of the donor. There are normally only approximately 200 recipients each month in New Zealand. The order of preference for selection based on the tissue type of the donor and recipient is:

HLA Identical

No mismatches at the HLA-B and HLA-DR loci.

Matching for cross reacting antigens.

Every attempt is made to avoid transplanting antigens present on the donor cells, not present in the recipient.

HLA antigens that were foreign on a previous graft are not transplanted on subsequent grafts.

iv) Clinical

Certain patients will be transplanted with an ABO compatible kidney, direct match negative, irrespective of the tissue typing results when the clinicians consider this is the only choice of treatment for the patient.

C. National Transplant Lists

Each month the Tissue Typing Laboratories at Dunedin, Christchurch, Wellington and Auckland distribute the latest serum samples from patients awaiting a kidney transplant to the other centres. At any time, each laboratory has on hand serum from all potential kidney recipients in New Zealand along with information of blood group, tissue type, HBsAg status, HLA antigens to avoid transplanting and HLA antibody percentage.

Based on the results of donor testing and information on all the potential kidney recipients, a list of suitable recipients nationally can be supplied to the clinicians.

Titration Standards

Alison Dent
Auckland Regional Blood Centre

This is an update to my article in the NZJ Med Lab Science Vol 46. No.3.

Salmond Smith Biolab have produced a new batch of Control Serum, CS 9225, Expiry July 1994.

When titrations are performed with doubling dilutions in AB serum and R_1R_2 indicator cells, a titre of 256 is consistently achieved with a variation of one 'tube' higher or lower acceptable.

Plasma Fractionation

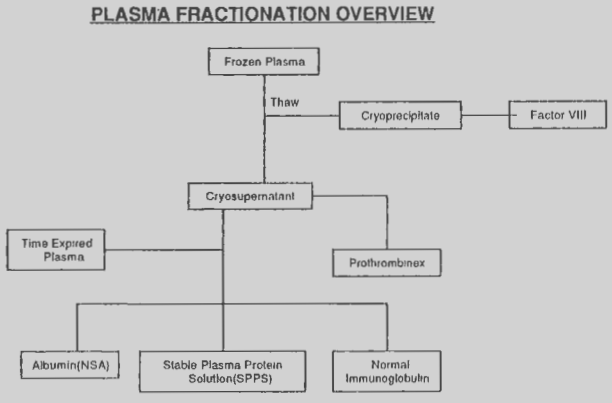
Alison Dent
Auckland Regional Blood Centre

Plasma Fractionation is a necessity as component therapy is regarded as the most cost effective method of utilizing our limited plasma resources. Component therapy is the use of a specific product to fulfil a patient's individual needs.

New Zealand produces some of its own plasma components, e.g. cryoprecipitate and some Factor VIII, however, Albumin (NSA), Stable Plasma Protein Solution (SPPS), immunoglobulin preparations, Prothrombinex and most of our Factor VIII are made at Commonwealth Serum Laboratories (CSL) in Melbourne, Australia. New Zealand frozen plasma is sent to CSL to be manufactured into requested product and returned to New Zealand.

The clotting factors, e.g. Factor VIII and Prothrombinex, are produced from cryoprecipitate and its supernatant by various protein fractionation techniques of which details will be dealt with in the next issue. Albumin (NSA), SPPS and the immunoglobulins (N Ig, Hep B Ig) are produced by Cohn Fractionation from fresh frozen plasma with the clotting factors being extracted as the first step. (Figure 1)

Figure 1



Cohn Fractionation originated in the 1940's after work of Dr E J Cohn[1] was published. Most plasma processed throughout the world is still fractionated following those guidelines. Modifications of those original methods have mainly been directed towards more economical production of good yields of those proteins (e.g. Albumin and SPPS) that are used in large quantities in clinical practice. Column chromatography is a newer method of plasma fractionation which is now also being looked at by such companies as CSL for the future production of plasma components.

Cohn Fractionation is a five parameter system based on the influence of the precipitation action of alcohol due to pH, ionic strength, temperature, protein concentration and alcohol concentration.

Separation can be carried out in two ways:

1. Conditions are chosen which will maximize the solubility of the desired protein, whilst minimizing the solubility of all the others.
2. The converse of "1" whereby the desired protein will be the one precipitated.

Ethanol is the most used precipitation agent in large scale production of the main plasma proteins. This is due to a number of properties such as its miscibility with water, relative chemical inertness, low toxicity, inexpensiveness and ease of availability.

Another advantage of significant importance in a fractionation process is its inhibition of bacterial growth and thus of pyrogen formation. This inhibition also results indirectly from the low temperatures needed when working with ethanolic protein solutions.

Cohn Fractionation of plasma components proceeds in a step by step fashion with alteration of one of the five parameters facilitating progression through to the desired end point. Principally, shifts in ethanol concentration and pH being of the utmost importance.

This progression is shown by the flow diagram which details production of Albumin, SPPS and the immunoglobulins. (Figure 2 — see overleaf).

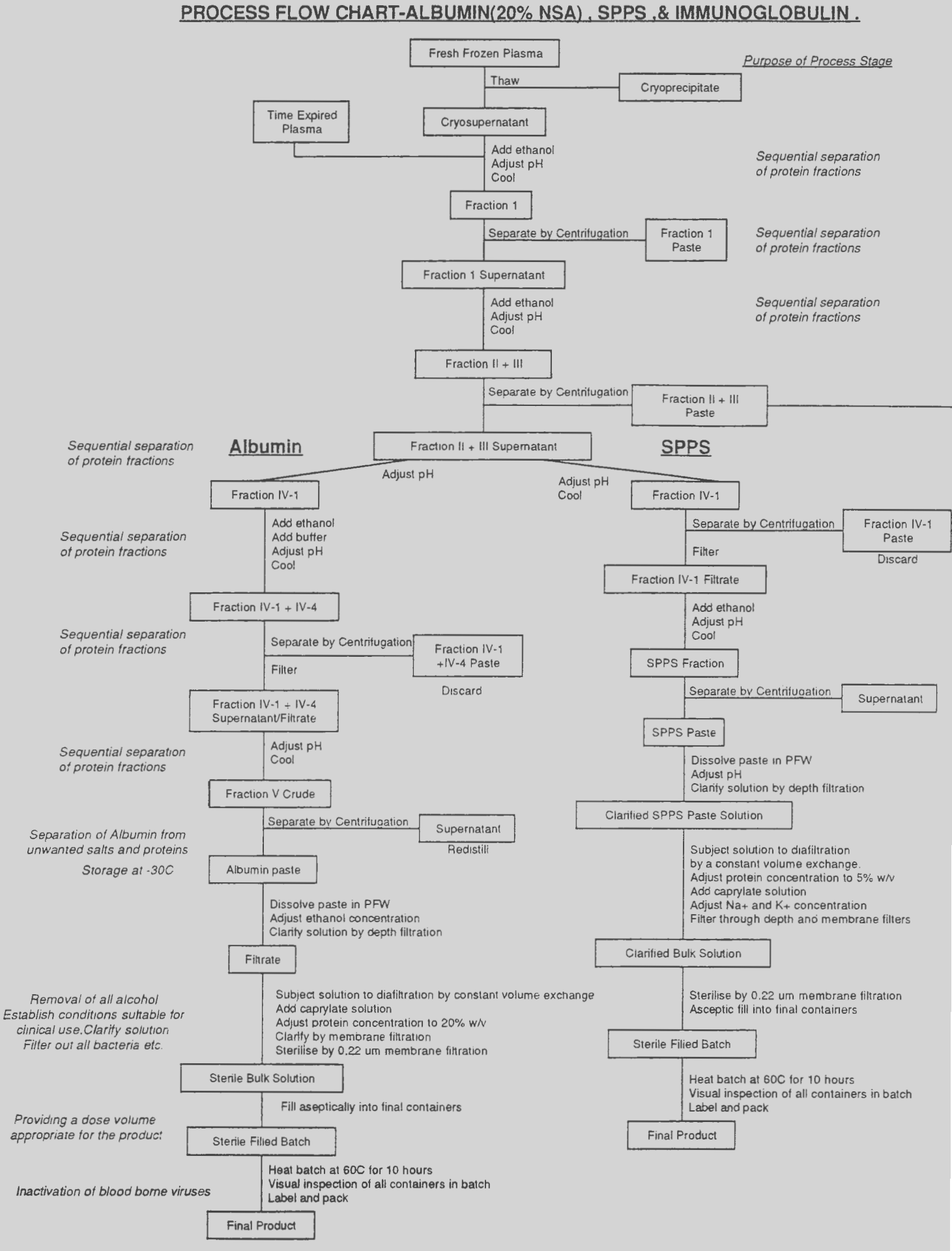
Specific intramuscular immunoglobulins are processed in the same way as normal immunoglobulin. The starting point however is with Hyperimmune Fresh Frozen Plasma specific to that of the desired end product, e.g. High Titre Anti-D Plasma for Anti-D Immunoglobulin. Also, there is a need to adjust potency and concentration. This occurs when the Freeze Dried Fraction II is dissolved in PFW and the thiomensal and glycine are added.

Reference

1. 1946 J.Am.Chem.Soc. 68, 459-475.

Figure 2

1992 Flow Diagrams of Plasma Fractionation at CSL.



Normal Immunoglobulin

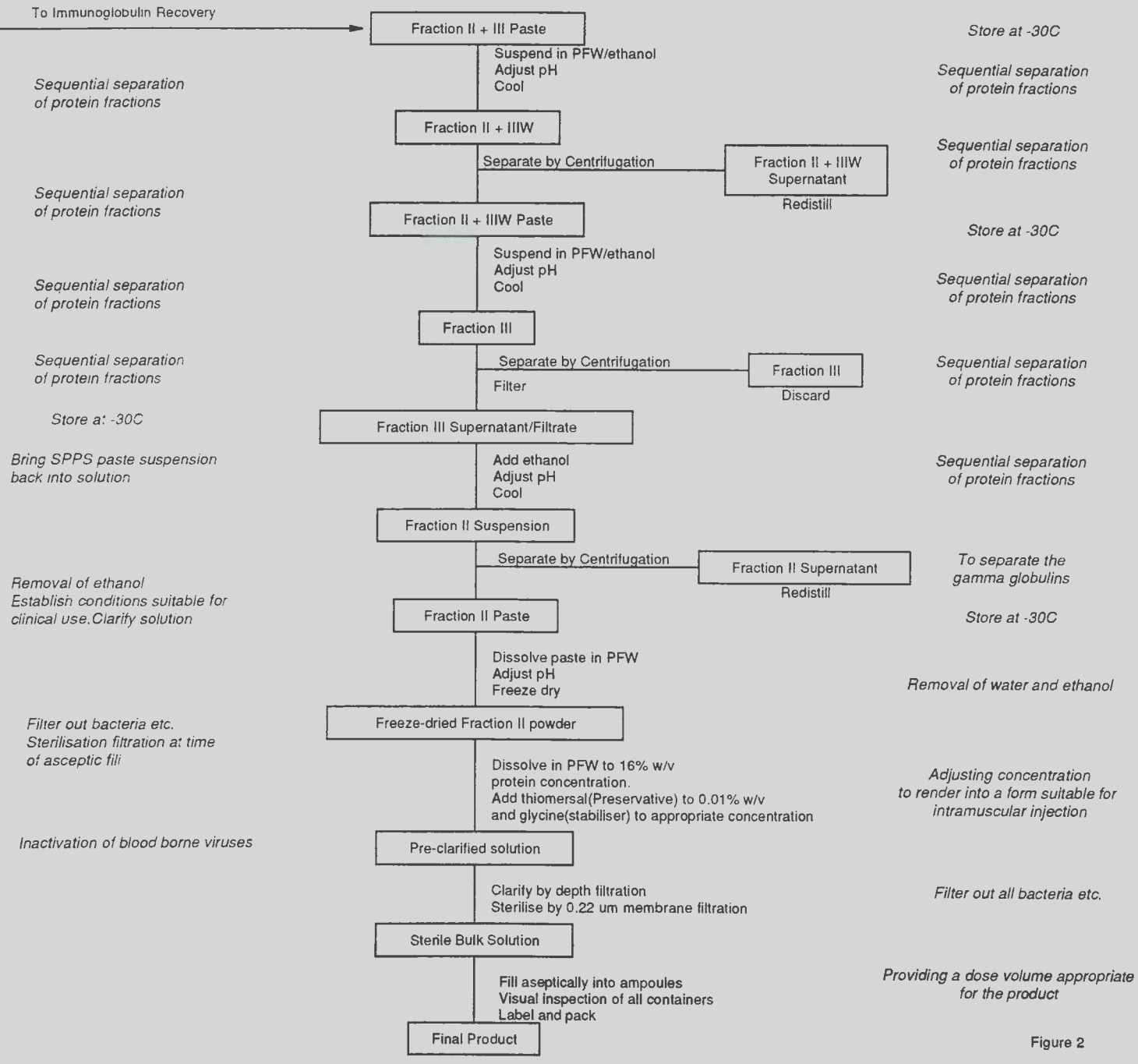
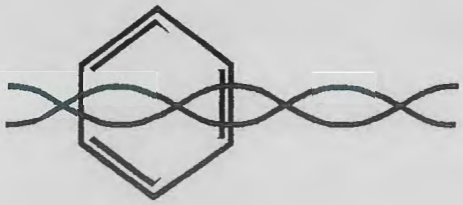


Figure 2



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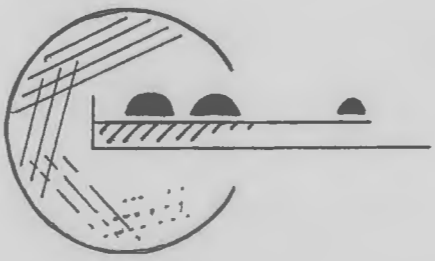

MICROBIOLOGY

SPECIAL INTEREST GROUP

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Editor
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Membership fees for the year beginning April 1, 1991 are:

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Members wishing to receive their publications by airmail should contact the Editor to make the necessary arrangement.

Membership Sub-Committee Report November 1992

Since the August meeting there have been the following changes:

	11.11.92	23.8.92	12.5.92	26.3.92
Membership	1244	1256	1188	1188
less resignations	7	27	6	3
less G.N.A.	-	19	-	6
less deletions	-	-	-	-
less deceased	-	-	-	2
less duplications	-	-	1	1
	1237	1210	1181	1174
plus applications	5	34	75	14
plus reinstatements	-	-	-	-
	1242	1244	1256	1188
Composition				
Life Member (Fellow)	12	12	12	12
Life Member (Member)	5	5	5	5
Fellow	20	20	20	21
Member	678	678	671	670
Associate	443	443	462	393
Non-practising	58	60	60	61
Honorary	26	26	26	26
Total	1242	1244	1256	1188

Applications for Membership
W. MIDDLEMISS, Timaru Hospital, C. MYERS, Dunedin Hospital, A. OAKLEY, Rotorua Hospital, P. WILLIAMSON, Dunedin Hospital, D. YEOMAN, Tauranga Hospital.

Resignations
D. LARSON, Christchurch, P. LINDSAY, A. MEADS, Hutt Hospital, A. NEMET, Overseas, R. OLSEN, Overseas, I. ORCHARD, R. TOLLEY, Taranaki.

NEW ZEALAND INSTITUTE OF MEDICAL LABORATORY SCIENCE 1993 CALENDAR

25/26 February	Council Meeting - Auckland
30 April	Committee Annual Reports to be with the Executive Officer
30 April	All accounts to National Treasurer for auditing
13/14 May	Council Meeting - Auckland
28 May	Applications close for Specialist Certificate examinations
28 May	Applications close for QTA examinations
31 May	Proposed rule changes and remits to be with the Executive Officer
26 June	Nomination forms for the election of Officers and Remits to be with the membership
1 July	Annual Staffing Survey
6/7/8 July	Fellowship examinations
17 July	Nominations close for election of Officers
5 August	Ballot papers to be with the membership
11 August	Annual Report and Balance Sheet to be with the membership
18 August	Ballot papers and proxies to be with Executive Officer
23/24 August	Council Meeting - Christchurch
25 August	AGM & SGM - Christchurch
25/26/27 August	Annual Scientific Meeting - Christchurch
2/3 November	QTA examinations
10/11 November	Specialist Certificate examinations
18/19 November	Council Meeting - Auckland

LETTERS TO THE EDITOR

Dear Editor,

We read with interest the article by Bluck and Blacklock on haematological parameters in normal babies (1). Their findings of no racial differences in haematological parameters is in contrast to other studies in New Zealand adults where racial differences have been found for the platelet count (2,3) haemoglobin and white cell count (3). They ask the question whether the racial differences in haematology parameters in adults are life-style generated rather than being racially controlled.

One such life-style factor could be body weight as it is known that the Maori are generally heavier than Caucasians (4) and that the circulating blood cells are positively correlated with body weight (2,5). However, body weight alone cannot explain totally the reported racial differences in the platelet count, as this parameter remained higher in Maori men compared to Caucasian men when weight was taken into consideration by analysis of covariance (2).

Thus until other life-style factors, such as diet, have been shown to affect haematological parameters, we believe that these reported racial differences in New Zealand adults (2,3) are in part genetic, in part life-style generated and in part due to as yet other unidentified factors. These racial differences may be of value as epidemiological markers for ischaemic heart disease (6), a condition over-represented in the Maori community (4). It would be of interest to re-examine Bluck and Blacklocks' data (1) to determine whether their findings of no racial differences in haematological parameters could have been confounded by the weight of the babies. For instance, if the Maori babies were lighter than the Caucasian babies, then any genetic differences in haematologic parameters would have been masked by the confounding effect that weight has on the circulating blood cells (2,5).

Yours sincerely

Robert Siebers
MIBiol, FNZIMLS
Department of Medicine
Wellington School of Medicine

John Carter
FRACP, FRCPA
Department of Haematology
Wellington Hospital

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NZJ Med Lab Science 1993; 47(1) : 38-39.

Polymerase chain reaction from an Immunohaematology viewpoint

Holly Perry

Protein Genetics Department, Auckland Regional Blood Centre.

The Polymerase Chain Reaction

The Polymerase Chain Reaction (1) (2) (PCR) is an invaluable tool in the area of DNA technology. The aim of PCR is to amplify a region of interest of DNA for further study. In this process, a specific region of DNA can be amplified a million fold (3). There are 3 steps to the reaction. First, DNA is denatured at high temperature to produce complementary single strands. Next, 'primers', or synthetic oligonucleotides are annealed to flank the target sequence to be amplified. The primers hybridize to complementary sequences in the genomic DNA at a relatively low temperature. The temperature is then raised to a point where the thermal stable enzyme Taq polymerase (4) is able to mediate the extension of the primers along the target sequence. Excess deoxynucleotide triphosphates (dNTPs) are added to the reaction mixture to facilitate the complementary pairing of bases Cytosine with Guanine, and Adenine with Thymine. There are now 2 copies of the template DNA where there was previously one. The temperature is again raised to denature the double strands, and the cycle begins again. An exponential increase in the region of DNA between the primers results. Typically, a worker might continue the reaction for 30 cycles. Thermal cycle instruments are available to automate the process.

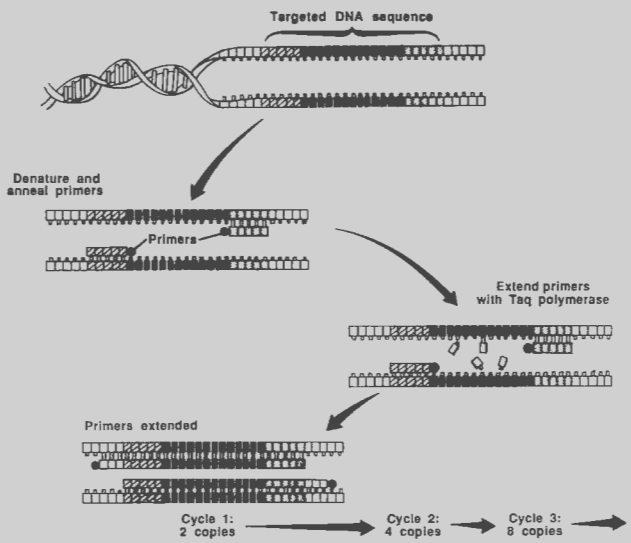

Figure 5-1. Amplification of DNA using the polymerase chain reaction (PCR). Each cycle consists of heat denaturation of the target molecules, annealing of an oligonucleotide primer to each target complementary DNA strand, and extension of the primers using Taq polymerase. The newly synthesized molecules are denatured to provide double the number of templates for the next cycle.

From DNA Fingerprinting: An Introduction, by Lorne T. Kirby. Copyright (c) 1990 by Stockton Press. Reprinted by permission of W.H. Freeman and Company.

Advantages of PCR

1. High degree of sensitivity and specificity for assays using PCR products. Very small amounts of the specific region of DNA of interest can be analysed after amplification.
2. Relative speed.
3. Wide choice of raw materials; tissue or body fluids.
4. Relatively simply sample preparation.

Disadvantages of PCR

1. Contamination

Aerosols are the major cause of contamination, which proves disastrous in the PCR area since DNA transferred by aerosol from one sample to the next will also be amplified in the Polymerase Chain Reaction. The exquisite sensitivity of the technique is also its major pitfall. Physical separation of the complete PCR system is necessary to ensure aerosols do not cause a problem and rigorous negative controls must always be included in the PCR batch (5).

2. Availability of the oligonucleotide primers is the key to the process, which means ongoing research is necessary to establish genomic libraries, e.g. for infectious agents. PCR is useless unless virus-specific sequences are previously established.

Direct applications of PCR in Immunohaematology

1. *Detection of Infectious Agents*

PCR technology is becoming increasingly important in the ability to recognise viruses transmissible by blood. Specific probes are used to detect amplified sequences of an actively replicating virus; giving a very sensitive and specific confirmatory test (6). It should be possible to eliminate the HIV "window period" in this fashion. (7) Hepatitis B and C, HIV 1 & 2, HTLV 1 & 2, CMV and other viruses are all agents where PCR could be of value in testing blood. (8) (9) (10) (11) (12) (13). However, the procedure is not yet practical for routine blood banking and would have to be automated before it could be used for screening blood for infectious agents.

2. *HLA typing*

Sequence specific oligonucleotides (SSO's) are being used to type many of the amplified products of the HLA loci as an adjunct to serological typing (14). This may improve graft matching and survival (15).

Many more histocompatibility loci are being identified with the advent of various DNA technologies (16) (17). The technology in this field is progressing at a great rate, with PCR at its centre (18).

3. *Forensics and Paternity Testing*

DNA "fingerprinting" has its basis in allelic variation due to differences in the number of repeat regions in vertebrate minisatellite DNA (19). This technology requires between 50 ng and 1 μg of undegraded DNA (20). If PCR is used to amplify the hypervariable regions of interest (21), much smaller samples can be used; an obvious advantage in forensic science. Restriction Fragment Length Polymorphism (RFLP), with single or multilocus probes, is also being used in paternity investigations. If PCR is used to amplify Variable Number of Tandem Repeats (VNTR) loci, the analysis can be much quicker because the procedure does not require hybridization with specific probes. (22) (23) (24).

Glossary

Anneal:	To join together, by base pairing of complementary sequences, two separated DNA strands.
Denaturation:	Action of producing single stranded DNA from double stranded, by the application of heat or strong alkalis.
*Hybridization:	The pairing of complementary strands of DNA, or RNA and DNA, derived from different sources.

*Locus (plural, loci):	A specific position on a chromosome
Multilocus:	Refers to a number of different loci. Hybridization with multilocus probes demonstrates the presence of alleles at many loci.
Nucleotide:	A unit of DNA, containing a base, either Adenine, Guanine, Cytosine or Thymine, joined to a deoxyribose-phosphate.
Oligonucleotides:	Synthetic sequences of nucleotides, used as PCR primers and probes.
*Primer DNA:	A short, perhaps 20-mer, oligonucleotide annealed to the 5' end of a DNA template. The primer provides an initiation point for addition of deoxyribonucleotides in DNA replication.
*Probe:	A single-stranded segment of DNA, or mRNA, capable of being tagged with a tracer, such as ^{32}P, and hybridized to its complementary sequence.
Restriction Fragment Length Polymorphism (RFLP):	Variation in the length of resistriction enzyme generated fragments.
Single-locus:	Refers to one locus. Hybridization with single-locus probes demonstrates the presence of alleles at one locus only.
*Tandem Repeat:	The end to end duplication of a series of identical or almost identical stretches of DNA. See VNTR.
*Variable Number of Tandem Repeats (VNTR):	The variable number of repeat core base pair sequences at specific loci in the genome. See tandem repeat. The variation in length of the alleles formed from the repeats provides the basis for unique individual identification.

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LEPROSY

Leprosy has long been one of the most dreaded afflictions. For centuries to be a leper was to be an outcast without hope. Leprosy has now become a treatable and manageable disease.

For over 50 years Leprosy patients in the South Pacific area were sent to the island of Makogi for treatment. Over 4,000 patients had been welcomed to its dreaded shores. In 1969 it was closed as an island that had served its purpose and the 83 remaining patients at that time were either repatriated to their island homes or sent to a new hospital in Suva.

The World Health Organisation is currently working with the Pacific Leprosy Foundation to completely eliminate Leprosy from the South Pacific area by the year 2,000. Each year a few new cases are detected. These days new cases can be completely cured within a relatively short period with multi-drug therapy. Elimination of the disease depends on early detection and adherence to multi drug therapy administration.

Carville, an internationally known research centre today, was once a prison-like environment for Leprosy patients. Today it is known in particular for research on the Leprosy bacilli. In all parts of the world many people have dedicated their lives to caring for the victims of Leprosy.

Sister Hilary Ross

Sister Hilary Ross was an outstanding woman who struggled at Carville for 37 years against Hansen's Disease.

The following is an extract from an article by Cynthia M. Gould from "The Star" Carville, Louisiana on the work of Sister Hilary Ross in the early days of Carville.

"When the Daughters of Charity arrived at Carville, Louisiana, in 1896, 31 patients with leprosy resided in dilapidated slave camps at the Indian camp plantation located 80 miles from New Orleans. The victims of this dreaded disease lived in a prison-like environment; those who tried to escape were returned to Carville in handcuffs like common criminals. Any patient who dared to journey to the Post Office, located in the tiny hamlet of Carville 2 miles away, was brought back by guards and thrown into jail for leaving without permission. Only patients with medical discharges or special passes could leave the hospital premises. There were no telephones for patient use, and emergency long distance calls to notify relatives of a surgical procedure or change in discharge plans prompted an escape to Baton Rouge through the "hole in the fence" which ripped through barbed wire surrounding the northern sector of the Institution. This dismal scene lasted for nearly 50 years until the discovery of the sulfone drugs which rendered the disease non-infectious in 1941.

Of the scores of missionary and medical personnel who laboured to eradicate the bacterium and the social stigma of leprosy, or Hansen's disease, one stands out as an exemplary figure, Sister Hilary Ross.

For 37 years this remarkable nun worked to eliminate the ignorance and misunderstanding at Carville through her humanity and compassion for patients, her role in the pharmacy and laboratory research during the sulfone era, and her contributions to professional organisations on both local and international levels. Although extensive literature exists on Hansen's disease, only newspaper clippings, scientific journal articles and short personal accounts of her life, pay tribute to this Daughter of Charity.

Sister Hilary's educational background covered many medical areas. While studying nursing, she underwent surgery for an opaque area around the ear which appeared on x-ray and evoked medical concern. This operation caused a marked facial nerve paralysis and her superiors removed her from the nursing curriculum. Thereafter, she studied pharmacy under the guidance of Sister Alphonsa Porter and enrolled in academic courses at the University of Wisconsin from 1919-1921. Because Carville needed a Pharmacist in 1922 she attended classes at Charity Hospital in New Orleans for 10 months and passed her State Board Examinations at Tulane University, which awarded her Certification in Pharmacy.

Later the medical staff needed a laboratory technician and made arrangements for Sister Hilary's additional education in the field of medical technology. A pathologist travelled from nearby Baton Rouge to teach her. She even enrolled in laboratory courses in St. Louis, Missouri from 1927-29. This educational background supplemented her Bachelor of Science degree, which she earned from Louisiana State University in 1937. Ultimately, Sister Hilary served her remaining years at Carville as a Research Biochemist (1940-1960) and Director of the Laboratory where she devoted many hours to research on a disease that perplexed physicians and successfully invaded every part of the body.

Hansen's disease is an infectious condition caused by the bacterium *Mycobacterium leprae*. It was first isolated by a Norwegian scientist, Dr. Gerhard Armauer Hansen in 1873. Typically, Hansen's disease consists of three types: lepromatous, tuberculoid, and dimorphous or borderline forms. The lepromatous type predominant at Carville, affects the skin first and the major nerves later. The tuberculoid type, prominent in Africa and Asia, exhibits less significant skin lesions, but invades and destroys the peripheral nerves of the arms and legs.

Sensory loss occurs in both forms. Under the microscope the dimorphous form resembles both lepromatous and tuberbuloid types and could develop into either form during the course of the disease.

Victims of the disease were regarded as outcasts and wore distinctive clothing with bells or clappers to distinguish themselves from healthy citizens and to warn them of their "infectious" nature.

Prior to the introduction of the sulfone drugs in the 1940's "inmates" at Carville lacked civil liberties and acquired classification with such infectious Asiatic diseases as bubonic plaque, smallpox, and yellow fever. Barbed wire surrounded their hospital dwelling and segregated the residents from the outside. Although Hansen's disease was less contagious than the common cold and significantly less infectious than tuberculosis, individuals stigmatised with the disease could not use public trains or buses.

Confronted with these advertisities, Sister Hilary fought against these prejudices by sharing her knowledge on Hansen's disease and treating the patients with compassion and understanding. Patients frequently came to visit her on the second floor laboratory where she explained pertinent diagnostic tests to them and boosted their morale.

Because discharge from Carville during pre-sulfone days required 12 bacteriologically negative exams, performed on a monthly basis, the patient often faced the demoralisation of

beginning all over again if the 12th exam returned positive.

The equipment and accommodation used by Sister Hilary lacked sophistication and space when she first arrived at the Pharmacy in 1922. A small cottage built in 1906, not only provided a meagre facility for the pharmacy but also doubled as a doctor's office and operating room.

Similar in construction to the crude pharmacy, the laboratory in 1929 also lacked space and sophisticated equipment. The patient's kitchen pantry served as the blood chemistry section, while wooden furniture provided a working area for general laboratory procedures and patient training purposes. To assist Sister Hilary in concentrating on blood chemistry determinations and research, patients learned the necessary skills to perform routine laboratory tests.

Relieved from the routine laboratory duties by the patient staff, Sister Hilary explored more technical aspects of the disease. She investigated biochemical elements of infected patients to help physicians establish therapeutic management and to satiasfy her own inquisitive, academic mind.

She questioned the blood changes which occurred in Hansen's disease after the absorption of the digits in the hands and feet, a phenomenon commonly mistaken for the "finger and toes dropping off". After examining x-rays Sister Hilary concluded that no definite relationship existed between the bone changes and serum calcium and phosphorous which comrpise the mineral content of the bones.

Promin treatment began at the United States Public Health Service Hospital in Carville on March 10th, 1941, when a Dr Frank McCreary injected six volunteer patients with the new experimental drug. Other pharmaceutical laboratories introduced new sulfones for oral administration. Abbott Laboratories developed Diasone in July 1943: Promizole entered the pharmaceutical market in March 1945. All the sulfone drugs required periodic urinalysis and blood counts to detect toxic effects on the body's blood components and kidney functions. Sister Hilary played a crucial role in the lab, working overtime to complete these routine lab procedures, and also performed experiments evaluating the absorption and excretion of the sulfone drugs in the blood and urine.

She worked closely with physicians and medical technicians who provided both scientific expertise and technical assistance during her laboratory research. Ultimately this research culminated in 46 scientific journal articles which she wrote or co-authored. All her research data required the rigid scheduling of specimen collection to eliminate unwanted variables that could falsify results.

Sister Hilary's career reached its zenith in 1958 when her work on Hansen's disease received distinguished awards which were coveted by leaders in the medical communnity.

Sister Hilary journeyed to St. John's University in Brooklyn, New York, where she accepted recognition for her work with Hansen's disease during the University's commencement exercises. The President of the University, Rev. John Flynn, had advised Sister Hilary that she had been selected by the Board of Governors to receive the President's medal - an award given to a woman for the first time in 88 years.

In 1958 she received her most prestigious credit when she received the Damian-Dutton Award. This award recognises individuals who have contributed assistance through education, science or humanitarian aid to those afflicted with Hansen's disease throughout the world. All of her work brought applause and awards from both the scientific and lay community during a time when men dominated science and medicine. For a woman and a nun to achieve such recognition was truly a remarkable feat. Although she was not a physician, her efforts, combined with concurrent medical research showed the world that the ancient scourge was not insurmountable and provided its victims with hope".

NEW PRODUCTS AND SERVICES

FOSS ELECTRIC A/S, NOW REPRESENTED BY RADIOMETER PACIFIC LIMITED.

The manager of Radiometer Pacific Limited Mr Laurie Powell has announced that Foss Electric of Denmark have recently taken up a financial interest in Radiometer Pacific Ltd based in Auckland. This is intended to establish a more direct link with the many existing Foss Electric customers throughout New Zealand.

Today Foss Electric A/s is the largest company in the world within its specialized field. Its name is synonymous with milk and dairy product testing.

Throughout its history, Foss Electric have pioneered new product concepts in the field of milk and food analysis. This emphasis on innovation is continuing in the 1990s with new instruments for automated and rapid testing of total bacteria count and specific bacteria — as well as second and third generation versions of the established products in the range.

Foss Electric have a commitment to continuous and substantial investment in both identifying market requirements and the research and development needed to satisfy those requirements. The company's strength and the key to its continuing success is its ability to combine different technologies — fine mechanics, electronic hardware and software, optics, chemistry and biochemistry — into analytical instruments which exactly match the needs of dairy and agricultural industries worldwide.

In support of Foss Electric's aims and achievements Radiometer Pacific Ltd have acquired highly skilled and experienced staff along with the necessary resources to provide full technical application and service support for the entire range of Foss Electric products in operation throughout New Zealand.

Mr Powell also stated that Radiometer Pacific's "Commitment to Excellence" will bring prompt, courteous and competent attention to all Foss customer equipment requirements.

Effective from 1st September, 1992 all Foss Electric Sales and Service inquiries should be directed to:

Mr David Whitley, or Mr Campbell McCracken, at Radiometer Pacific Ltd, on Telephone: (09) 573 1110 or Facsimile: (09) 573 1106.

RADIOMETER ADDS A NEW DIMENSION TO THE DEEP PICTURE ™OF BLOOD GAS ANALYSIS.

Radiometer is now introducing the new ABL™505 combined Blood Gas and Electrolyte Analyzer. The ABL505 measures pH, pCO_2 pO_2, cK^+, cNa^+ and cCa^{2+} or cCl^- going one step beyond the traditional blood gas analysis to also include electrolytes. As an added plus, the ABL505 can interface with the OSM™3 HEMOXIMETER™ which measures *ct*Hb, sO_2, FO_2Hb, *F*MetHb, *F*COHb and *F*RHb. When you couple the ABL505 and the OSM3 you have a comprehensive STAT profile when treating the critically ill and you get an invaluable expansion of The Deep Picture.

The ABL505 and OSM3 system provides precise data concerning the acid-base status and blood electrolytes. It also provides data which describes the capabilities of the patient's uptake, transport and release of oxygen to the tissues, in other words — The Deep Picture. The ABL505 and OSM3 system requires only 160μL of whole blood and delivers all results in only 54 seconds.

The ABL505 data management facilities utilize all results from both the ABL505 and OSM3. That, of course, makes the analyzer easier to handle, enabling you to concentrate on the results and not the handling of the analyzer.

For detailed product information concerning the ABL505 and OSM3 please contact:

Radiometer Pacific Ltd, P.O. Box 12-416, Auckland, New Zealand. Telephone: (09) 573 1110, Facsimile: (09) 573 1106.

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HANDY PIPETTE TIP REFERENCE GUIDES

Now available from Salmond Smith Biolab are two easy reference pipette tip charts designed especially for the customer to make pipette tip ordering easier.

A chart is available for both the Laboratory and Research grade pipette tips to cater for varying pipetting requirements.

Popular agencies of Multi, Biosep, LPI, Volac are listed with tip features and are cross referenced against various pipettor brands e.g. Eppendorf, Gilson, Finn.

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SIGMA DIAGNOSTIC REAGENTS

Sigma Diagnostics has chosen to commit resources and research to perfecting reagent formulations that work well on Hitachi and all other popular analysers. Information is available on the linearity and amazing stability of the various types of reagents.

Changing chemistries from one manufacturer's reagent to another can be a real hassle, but Sigma makes it easy with —

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— parameters for particular analysers

Contact your Biolab Scientific representative for details.

KODAK NEW ZEALAND LIMITED: CLINICAL DIAGNOSTICS DIVISION

Kodak New Zealand Limited is pleased to announce the establishment of their New Zealand Clinical Diagnostics Division head office being located in Parnell, Auckland.

Kodak Clinical Diagnostics is internationally renowned as the benchmark for the provision of quality Health Care products to the in-vitro diagnostic market. This enviable reputation is based on the Ektachem range of Biochemistry Analysers, using unique dry-chemistry technology.

Kodak's commitment to the Health Care Market has now been further enhanced with the acquisition of the Amersham International Immuno-diagnostics Division; the Amerlex and Amerlite range of products are well known and accepted by the majority of laboratories in New Zealand.

The acquisition of Amersham's Immuno-diagnostic Division began in November 1990 with the formation of a 50-50 joint venture with Kodak, called Amerlite Diagnostics Limited. Amersham wanted to develop an automated immunoassay system but needed further expertise and a large capital investment; at the same time, Kodak was looking to build its success of their Ektachem, biochemistry analysers and ways of broadening their product range as a service to their customers. Due to the unparalleled success of the joint venture, Kodak decided to exercise its option for 100% of Amerlite Diagnostics Limited in March 1992.

This was the date of the official formation of Kodak Clinical Diagnostics, which saw the amalgamation of two centres of excellence providing R & D and manufacturing.

The main product lines manufactured and supplied by Kodak Clinical Diagnostics now include the Ektachem and DT series of clinical chemistry analysers, Sure Cell test kits, Amerlex test kits (radio immunoassay) and Amerlite instrumentation and reagents (non radio immunoassay).

The Amerlex and Amerlite product range has been marketed and supported by Amersham New Zealand, a subsidiary of Amersham Australia and this business has been transferred to Kodak New Zealand Limited.

"With the introduction of the Ektachem range of Biochemistry analysers to the New Zealand market, this merger will certainly carry benefits for the customer" said Mr John Allen, Managing Director of Kodak New Zealand Limited, "as our aim is to excell in quality, quality products, quality service and quality technical support."

"Kodak intend to be the recognised global leader in the invitrodiagnostics market by providing customers with innovative products and services of the highest value to improve the quality of health care" says Mr Allen.

The Kodak New Zealand Limited Clinical Diagnostics Division is represented by the following team members . . .
Mr John Allen, Managing Director, Ph. 377 8950 Ext. 8819; Mr Malcolm Hale, Manager, Logistics & Support Services, Ph. 377 8950 Ext. 8817; Mrs Paula Lindsay, Technical representative, Ph. 377 8950 Ext. 8724; and Mr Doug MacLeod, Customer Relations & Product Planning, Ph. 377 8950 Ext. 8706.

NEW PRODUCT RANGE

Kodak Clinical Diagnostics is now proud to offer pathologists and medical scientists an even broader range of quality diagnostic testing products including Ektachem in-vitro diagnostics, SureCell rapid test kits, and Amerlite and Amerlex Immunodiagnostics.

Ektachem Products

The Ektachem analyzers and slides for in-vitro diagnostic testing have made Kodak a familiar name in clinical chemistry. More than 15,000 unique dry-chemistry Kodak Ektachem analysers, with their fast test turn-around, are currently in use in 55 countries across the globe.

Rapid Test Kits

Kodak SureCell test kits provide fast and highly accurate results for a selection of conditions where rapid, on-the-spot results are advantageous. Each kit is self contained with a supply of test cells and all necessary reagents. Furthermore, no incubation period between reagents is necessary in the smooth-flowing procedure.

Immunodiagnostics

With the recent acquisition of the immuno-diagnostics business of Amersham International, Kodak's range now extends to Amerlex and Amerlite reagents and instrumentation systems. The range of reagents cover thyroid testing, reproductive and fertility testing, anaemia and hepatitis testing.

For further information on Kodak Clinical Diagnostics Products please contact: Paula Lindsay, Clinical Diagnostics Division, Kodak (New Zealand) Limited, PO Box 2198, AUCKLAND. Ph 377 8950.

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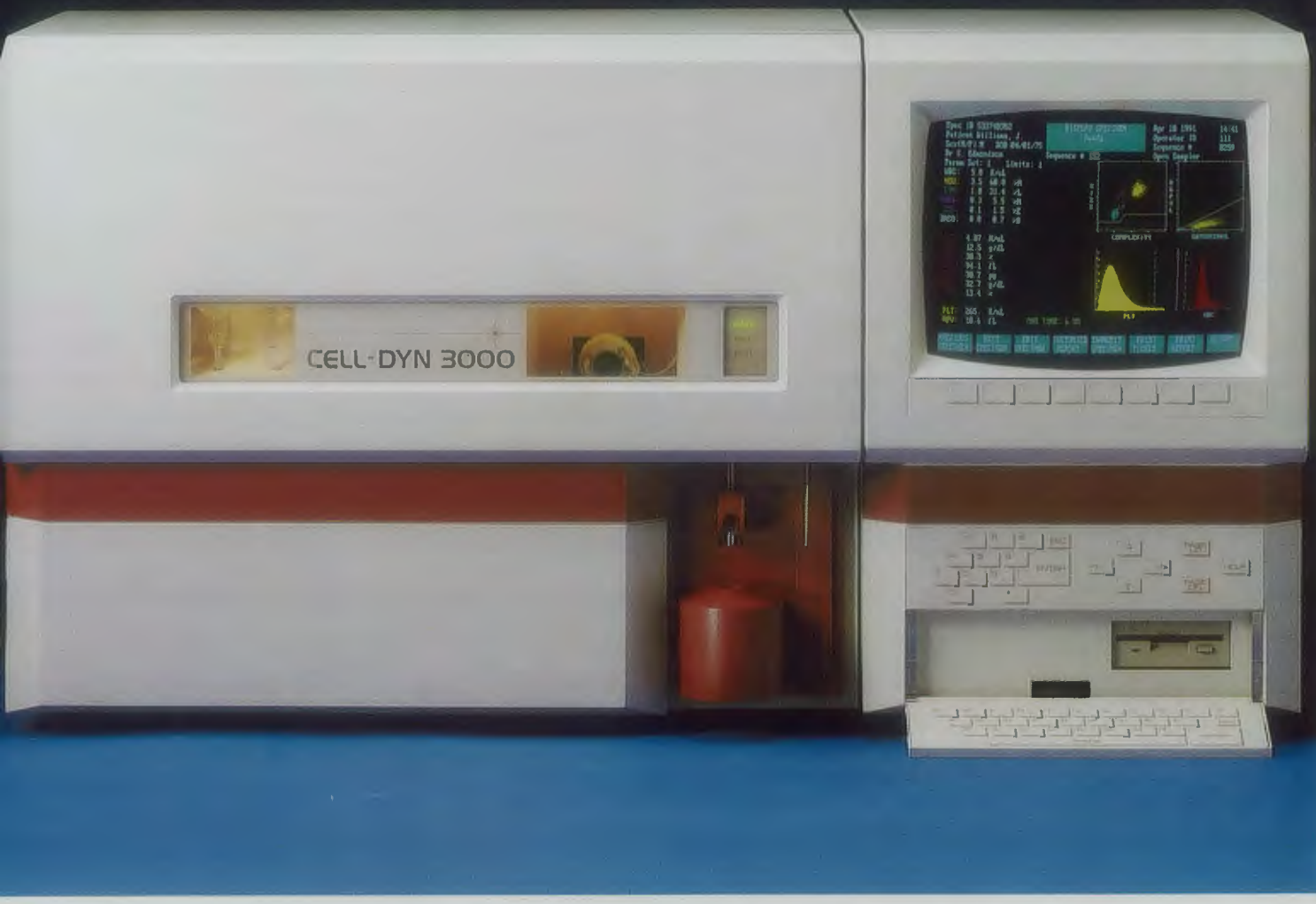

For Further Information Contact Warren Mitchell

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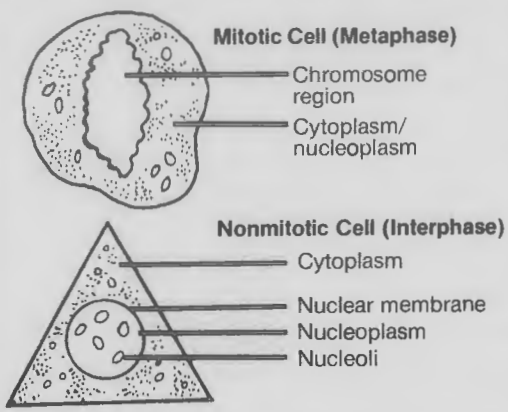
Well accepted and in use in the NZ market, Immunoconcept's ANA Test System is available in both fluorescent and non-fluorescent Colorzyme™ Kits and slides.



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