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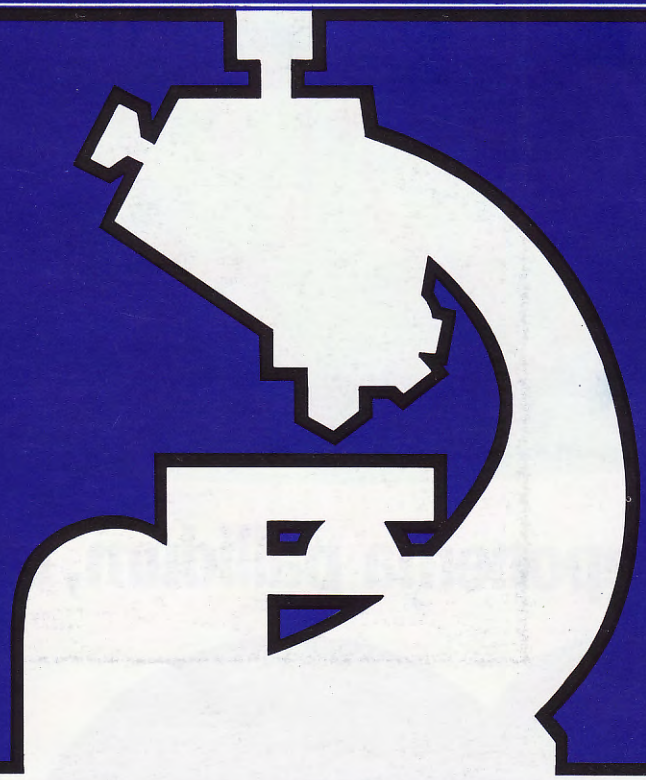

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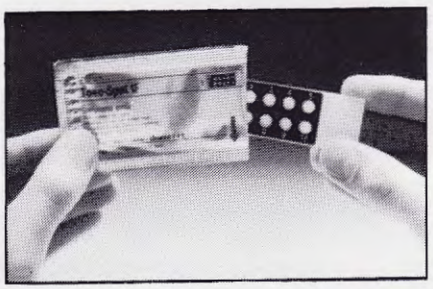

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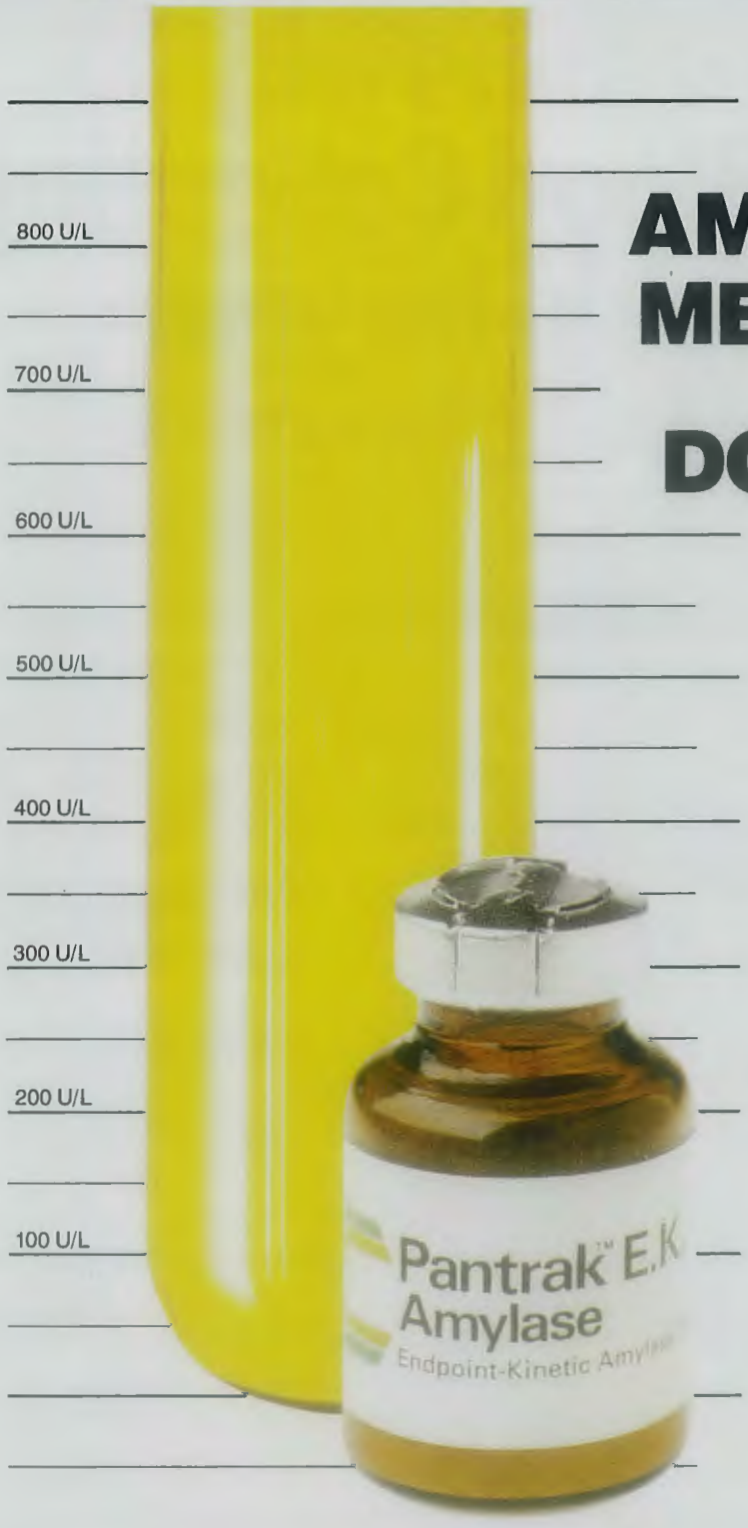

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The Application Of Cell Marker Studies To The Classification Of Acute Leukaemia — The Auckland Experience

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Running Title: Cell marker studies in acute leukaemia.

Abstract

Cell marker studies (CMS) are an important component of acute leukaemia classification, providing both information pertinent to selection of therapy and interesting insights into leukaemia cell derivation.

CMS have been performed on 127 patients with acute leukaemia. In 68 patients (54%) CMS made a major contribution to classification; they were particularly helpful in confirmation and subclassification of acute lymphoblastic leukaemia (ALL). The presence of markers of both myeloid and lymphoid type has been documented in 8 patients; in 5 of these there appeared to be a dual leukaemic population.

A scheme for the rational selection of antisera is described which uses 9 commercially available antisera (8 monoclonal antibodies and 1 conventional antiserum). This protocol enables patients to be classified into four therapeutically significant subtypes of ALL and two subtypes of acute myeloid leukaemia (AML).

Introduction

In the past the all important classification of acute leukaemia which determines subsequent treatment has been made according to the morphology of the blast cell and its specific cytochemical staining. Recently the use of cell marker techniques have been developed and have become an important component of the classification of acute leukaemia[1,2,3].

The term cell markers refers to a variety of antigenic substances present on the cell surface, in the cytoplasm or in the nucleus which relate both to the origin of the cell and to its level of maturation. Most of these have no known biological function.

Figure 1 shows the myeloid stem cell and its development into monocytic and granulocytic progeny. Only a limited number of myeloid cell markers can presently be identified on these by commercially available antisera; those measured in our laboratory are shown.

The monoclonal antibodies MY7 and MY9 (Table 1) identify antigens on all myeloid cells through to the more mature cells of both series. Only monocytic cells are identified by the MY4 antibody; and Ia antigen, (also known as the HLA-DR antigen) is seen on all stages of the monocytic series, but is found only on the blast cells of the granulocytic series. In a case of acute leukaemia, positivity with the MY7 and MY9 antibodies on the blast cells confirms the myeloid origin. If there is MY4 positivity also, this is strongly suggestive of a myelomonocytic derivation[4]. Early lymphoid development through to blast cell stage is shown in Figure 2. The pluripotent stem cell gives rise to both the myeloid and lymphoid cell lines. The earliest recognisable lymphoid cell, which bears the nuclear antigen terminal transferase (TdT), and surface Ia-like antigen, differentiates into the B cell series, and probably the T cell line[5].

Of the cells that differentiate along the B lymphocyte pathway, the earliest recognisable cell is called the pre-B cell; it corresponds to the level of maturity of lymphoid blasts seen in the most common type of acute lymphoblastic leukaemia (cALL). In addition to the persistence of the Ia antigen and the nuclear TdT staining, this pre-B cell has acquired two new antigenic substances — the common ALL antigen (identified by the cALL or J5 antibody) and the B1 antigen. Subsequent development gives rise to an early B cell, corresponding to the level of maturity of the cells of the Burkitt's or B cell ALL. Surface membrane immunoglobulin can now be detected, and the B1 antigen persists; nuclear TdT is usually not detectable at this stage.

In Figure 2 the T lymphocyte lineage is shown deriving from the most primitive lymphoid stem cell. This origin is by no means certain though early T cells demonstrate TdT, seen in the primitive

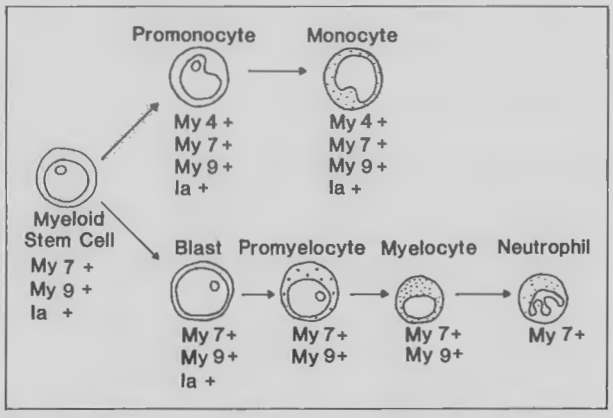

Fig. 1. *Distribution of antigens on normal bone marrow myeloid cells.*

lymphoid cell. Apart from this marker, however, T cells have a completely different set of marker substances which include many different antigens which relate to level of maturity and to function. Antibodies to 2 surface antigens are helpful in our work: the T2 antibody identifies T cells at all stages and the T11 antibody reacting with the receptor for sheep red blood cells (SRBC), which is variably expressed in early T cells, and is commonly present in mature T lymphocytes.

This report presents the results of cell marker studies performed on 127 patients with acute leukaemia. Due to the increasing number of monoclonal antibodies now available, a selective approach has been necessary. A protocol is described for phenotyping blast cells in patients with acute leukaemia. This uses commercially available antisera, and minimises the number of tests, aiming to classify leukaemia to the level necessary for selection of therapy and predicting outcome.

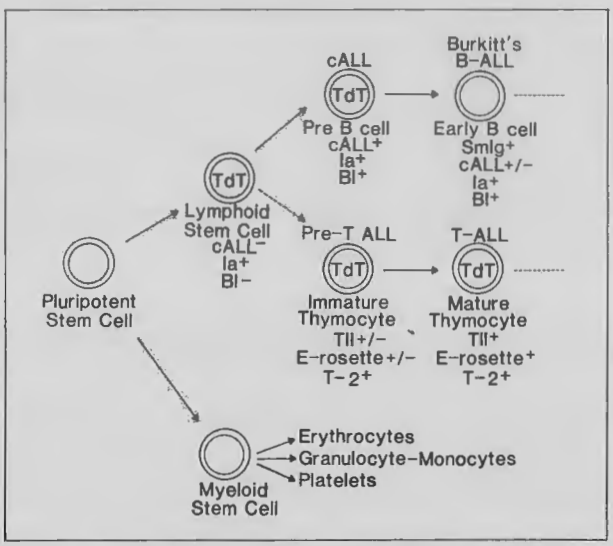

Fig. 2. *Distribution of antigens on early lymphoid cells and their relationship to lymphoid leukaemias.*

Table 1 *Antisera used in Testing*

Antisera	*Specificity*	*Source*
I2*	Ia-like (HLA-DR) antigen	Coulter Electronics
J5*	Human common acute lymphoblastic leukaemia antigen	Coulter Electronics
B1*	B1 associated antigen on human B cells	Coulter Electronics
T11*	E rosette receptor antigen	Coulter Electronics
MY4*	Normal and leukaemic human myeloid cells	Coulter Electronics
MY7*	Normal and leukaemic human myeloid cells	Coulter Electronics
MY9*	Normal and leukaemic human myeloid cells	Coulter Electronics
T2*	Human T cell antigen	Dakopatts
Goat F $(ab)_2$ anti-mouse immunoglobulin-TRITC	Mouse IgG (G+Lch)	Tago, Burlingame California
Rabbit anti-TdT	Rabbit antibody to calf TdT	Bethesda Research Laboratories (BRL)
Goat $F(ab)^2$ anti-rabbit immunoglobulin-FITC	Rabbit IgG	BRL
Swine anti-rabbit immunopglobulin	Rabbit immuno-globulin	Dakopatts (PAP Kit)
PAP, rabbit	Rabbit anti-peroxidase complexed with horseradish peroxidase	Dakopatts (PAP Kit)
Goat anti-mouse immunoglobulin-G30	Antibody to mouse IgG linked to 30nm gold particles	Janssen Life Sciences Products, Beerse, Belgium.

* Monoclonal antibody

Materials And Methods

Patient Population

Over a 2½ year period 127 patients both children and adults with acute leukaemia have had bone marrow and/or peripheral blood tested for cell markers, in addition to the usual morphological and cytochemical studies. Each patient was assessed for the contribution made by the CMS to the classification of the leukaemia, over and above the classification according to the conventional methods.

Laboratory Procedures

1. Cell Separation

Mononuclear cells from bone marrow aspirate or peripheral blood are separated by Ficoll-Hypaque (Sepalymph, Teva Pharmaceutical Industries Ltd, Jerusalem, Israel) density gradient centrifugation at 400g for 30 minutes[6]. Cells are washed in phosphate buffered saline pH 7.4 (PBS) and resuspended in Hanks medium containing 10% foetal calf serum (H-FCS) to a concentration of 20×10^6 cells/mL. Cytocentrifuge preparations are made with a Shandon Cytospin 2 (500 rpm 10 minutes).

2. Antisera

Details of the antisera used and their source is outlined in Table 1.

3. Controls

Cells frozen in liquid nitrogen from patients with known positive populations are included with all test protocols. Cytocentrifuge preparations frozen at -70°C were found to be particularly suitable TdT controls.

4. Indirect Immunofluorescence Assays

Surface membrane antigens are detected by indirect immunofluorescence using monoclonal antibodies and a secondary antibody labelled with tetramethyl-rhodamine-isothiocyanate (TRITC). Terminal transferase, a nuclear antigen is routinely assayed by indirect immunofluorescence using cytospin slide preparations with the second antibody labelled with fluorescein isothiocynate (FITC).

(a) *Monoclonal Antibodies.* The Coulter antisera are diluted and stored at -20°C according to the manufacturer's instructions. 100μL of this diluted antiserum is incubated with 50μL (10^6 cells) of cell suspension for 30 minutes at 4°C. After washing three times in PBS, pH 7.4 containing 2% foetal calf serum and 0.02% sodium azide (PBS-FCS) the cell pellets are resuspended in 50μL of optimally diluted Goat $F(ab)_2$ anti-mouse IgG conjugated with TRITC; these are incubated a further 30 minutes at 4°C. The cells are washed again and resuspended in 50μL of PBS-FCS. A drop is placed on a clean slide, coverslipped and examined for fluorescence with a Nikon Optiphot microscope equipped with an episcopic fluorescence attachment and a filter for TRITC.

(b) *Terminal Transferase.* The test was carried out using the BRL Terminal Transferase Immunofluorescence kit. Briefly, cytocentrifuge slides are fixed in methanol for 30 minutes at 4°C, rehydrated in PBS, and incubated in a moist chamber with 15μL of rabbit anti-TdT for 30 minutes at room temperature. Slides are washed in PBS with stirring, followed by a further incubation with FITC conjugated Goat $F(ab)_2$ anti-rabbit IgG. The slides are washed again in PBS, mounted in 50% glycerol in PBS and examined for nuclear fluorescence with a suitable filter for FITC.

(c) *Double Immunofluorescence Labelling.* Double immunofluorescence labelling two antigens in the same preparation, can be performed readily where the two primary antibodies are made in different species[7], or are of different immunoglobulin class or subclass. By using different fluorescent labels on the secondary antibodies it is possible to determine whether two separate populations are present or whether both antigens are present on the same cell. For example a murine monoclonal antibody can be used in conjunction with the rabbit anti-TdT antibody. Cells are incubated with a monoclonal antibody according to the procedure described above. After a wet preparation is examined for fluorescence, a sample is taken from the tube and diluted for cytocentrifugation. The assay for TdT is then carried out in a routine way. The preparations are examined alternately for the membrane antigen labelled with TRITC (red) and TdT with FITC (green). The two labels cannot be visualised simultaneously, because they are demonstrated at different wave lengths.

5.Immunoperoxidase Technique for TdT

The peroxidase antiperoxidase (PAP) technique for TdT is helpful where material available is limited, such as bone marrow smears or trephine trails; in this situation the immunofluorescent technique is complicated by background staining. Slides are fixed in methanol for 30 minutes at 4°C, rehydrated in PBS and sequentially incubated with the following reagents: normal swine serum (Dako), rabbit anti- TdT (BRL), swine anti-rabbit IgG (Dako), PAP (Dako). The preparations are washed in PBS in between incubation steps. Peroxidase activity is demonstrated by incubating the slides for 10 minutes at room temperature in the dark, in the following substrate: 3,3 diaminobenzidine tetrahydrochloride (0.6mg/mL) in Tris-HCl buffer pH7.6 containing hydrogen peroxide to a final concentration of 0.03%. The preparations are counterstained with Gill's haematoxylin[8] for 5-10 minutes.

6. Immunogold Staining (IGS) for Surface Antigens

Antibodies can be conjugated to gold particles which are visible with the light microscope[9]; positivity can be detected together

with morphological detail, often enhanced by specific cytochemical stains, such as non-specific esterase or peroxidase.

25μL (5×10^5 cells) of cell suspension are incubated with 50μL of diluted monoclonal antibody for 30 minutes at 4°C, washed twice in PBS containing 1% albumin, 1% human AB serum and 0.2% sodium azide, and resuspended in 15μL of goat anti-mouse IgG-G30. Tubes are left at room temperature for 30 minutes and shaken every 10 minutes. After further washing, the cell pellet is resuspended in 1mL of the wash buffer and cytocentrifuged. These preparations can be stained with Leishmans, for non-specific esterase[10], myeloperoxidase, or for TdT by the PAP method[11].

7. E Rosettes

This technique is not part of routine studies, but is used in mixed marrow populations, or when a dual blast population is suspected. Cells forming spontaneous rosettes with SRBC are assayed using neuraminidase pre-treated SRBC[12]. Cytocentrifuge preparations, made by spinning at 500 rpm for 2 minutes, are then stained with Leishmans, for myeloperoxidase activity or TdT-PAP.

8. Selection of Testing Methods

Where adequate cell suspensions are available, all testing is done by indirect immunofluorescence. Where limited material, such as bone marrow trephine trails or smears are available, immunofluorescence testing is difficult per se and tests for surface membrane antigens are fraught with technical problems. In these cases, TdT-PAP is one of the few reliable tests; because of its presence in the nucleus, it is not prone to the inaccuracies seen with surface membrane antigens. Unfortunately, the morphological detail of the TdT-PAP positive cell may be distorted by this staining.

In a number of situations, the double-staining techniques are desirable:

(i) When morphological appearances or marker studies suggest dual blast populations or mixed lineage of blasts.
(ii) Where the leukaemic population forms only a small part of the total marrow cells.

Here the test selection depends on the nature of the two populations. There are three possible combinations:

(i) Double indirect immunofluorescence.
(ii) Immunogold staining with subsequent cytochemistry or TdT-PAP.
(iii) E rosettes with cytochemical staining or TdT- PAP.

As the number of monoclonal antibodies available has continually increased over the study period, not all patients have been tested with all the antibodies listed in Table 1. This has been particularly so for myeloid antibodies, which have been in use for less than half of the test period.

A protocol delineating our present testing strategy is given as an Appendix.

Results

As a result of combined morphological, cytochemical and cell marker studies on 127 patients with acute leukaemia 62 patients were classified as AML and 53 as ALL; in addition there were 5 cases of mixed leukaemia, 2 of undifferentiated leukaemia, 1 of plasma cell leukaemia, and 4 cases who could not be classified, with methods available at the time of testing.

In 54% of the 127 cases, the CMS made an additional contribution to the information obtained from morphological and cytochemical studies. This contribution usually involved a more accurate subclassification of the type of leukaemia, which in some cases had a direct influence on the treatment protocol selected. The contribution of CMS was found to be greater in ALL than AML (Table 2).

Of 62 cases designated AML (which included all six of the variants of the FAB classification[13] and one case of megakaryoblastic leukaemia) in only 15 did cell marker studies aid this classification or add further information. Before the specific myeloid antibodies were available, in 8 cases with indeterminate peroxidase stains and morphological features, the absence of TdT was used as evidence against a lymphoid origin of the leukaemia. Since specific myeloid markers have been in use, in one case a straightforward AML, M1 ($MY7^+$ $M9^+$ $MY4^-$) was confirmed when cytochemistry and morphology made it difficult to decide between M1 or the myelomonocytic (M4) variant. In 2 patients the reverse applied, the MY4 positivity confirmed the myelomonocytic nature of the leukaemia. Three further patients had quite definite myeloid markers and cytochemistry but also showed positivity for TdT in a proportion of cells, so-called lineage infidelity.

Table 2 *Value of Cell Marker Studies in Leukaemia Classification*

Type of Leukaemia	Number of Patients	CMS Contributory	%
AML	62	15	24
ALL	53	44	83
Mixed	5	5	100
AUL	2	2	100
Other	5	2	40
	127	68	54%

AUL = Acute undifferentiated leukaemia

In contrast to the patients with AML, CMS were found to be helpful in the majority of patients with ALL (83%). Their contribution was both in excluding AML and in delineating the 4 subclasses of ALL (Table 3). The 4 subclasses are listed in order of prognosis with common ALL (cALL) having the most favourable prognosis[5]. Of the subclasses only the morphology of the uncommon Burkitt's or B cell acute lymphoblastic leukaemia is characteristic; the morphological and cytochemical distinction is less secure for the other variants, hence the value of the CMS, in 100% of the other subtypes.

It was found that a range of markers was necessary to confirm T cell ALL, due to the heterogeneous nature of CMS in this leukaemia[3,5]. Table 4 shows that 4 of the 8 patients with T cell ALL did not demonstrate the classical phenotype (seen in patients 1-4). In all 8 patients, however, the T2 antibody (probably the same as WT1[15]) was positive. Designation of T-ALL is important due to the different treatment protocol given to this prognostically poor group.

Five patients were defined as mixed acute leukaemia and would not have been adequately diagnosed without CMS. These included a patient with mixed T ALL/AML, a case of Philadelphia positive acute leukaemia with both myeloid and common ALL blast populations, and 2 patients with both null-ALL and AML populations. The fifth patient demonstrated both lymphoid and myeloid antigens on all the blast cells.

Table 3 *Subclassification of Acute Lymphoblastic Leukaemia*

Type	Number of Patients	CMS Contributory	%
ALL — not subclassified	15	8	53
Common ALL	20	20	100
Null ALL	6	6	100
T ALL	8	8	100
B ALL	4	2	50
TOTAL	53	44	83%

Discussion

Cell marker studies have made an important contribution to classification of acute leukaemia in our unit.

As expected, the contribution has been less important in cases of acute myeloid leukaemia, where morphological features alone are often diagnostic, and where cytochemistry, particularly the peroxidase stain is positive, by definition in M1 to M3 variants. In this group the contributions were both in excluding acute lymphoblastic leukaemia and in confirming/excluding the M4 variant where peroxidase staining is more variable. The finding of myeloid markers, particularly MY4, was helpful in a number of cases, both confirming myeloid origin, to enable treatment selection, and delineating whether the monocytoid sub-type was present with its particular clinical problems. Had specific myeloid antibodies been available throughout the whole period of evaluation a greater contribution of CMS may have been noted in

Table 4 *Expression of Antigens in T Acute Lymphoblastic Leukaemia*

Patients	TdT	E rosettes	T11	Ia	cALLA	T2/WT1
1-4	+	+	+	-	-	+
5	+	-	+	-	-	+
6	+	-	±	-	-	+
7	+	+	±	-	±	+
8	+	-	+	-	+	+

AML patients. The significance of TdT positivity in AML is not certain. Some feel it may delineate a group of patients with a different prognosis[16,17]. We feel it is helpful to identify this small group.

In contrast to the AML cases CMS were important in classification of the majority of patients with acute lymphoblastic leukaemia, making a contribution to confirmation or subclassification of the disease in 83% of cases. Of the remaining 17%, for whom marker studies were not found helpful, the majority were patients tested early in the evaluation period when TdT was the only lymphoid marker assessed, and this positivity was felt not to add to the morphological diagnosis. In addition, there were 2 patients with B-ALL who had the classical blast morphology, for whom CMS added no further information.

None of the patients with mixed leukaemias could have been diagnosed adequately without marker studies, of which the double labelling techniques were particularly helpful, both in demonstrating the double blast population, and in determining responses of blast populations to treatment. It is likely that the occurrence of mixed leukaemias may be found to be more common than was known prior to CMS. This should provide interesting insights into leukaemic cell derivation.

Appendix

Test selection: a current protocol.

Because of the cost of antisera and the amount of technologist time involved in CMS, a protocol has been derived to rationalise their use. The aim of this protocol (Table 5) is to (sub)classify acute leukaemia to the level necessary for selection of treatment and determination of prognosis, namely myeloid/monocytoid variants of AML and the four subtypes of ALL (null-ALL, cALL, T cell ALL, B cell ALL).

The initial step of this test selection is a myeloperoxidase stain to enable FAB classification. Where this is unequivocally positive, limited testing with myeloid markers, Ia-like antigen and TdT is undertaken. The TdT is included so that cases with mixed blast populations of myeloid/lymphoid type will be detected, and to define cases of acute myeloid leukaemia with TdT positivity.

Where the peroxidase stain is negative or equivocal, 7 antisera are used for further testing. This ensures that monocytoid variants of AML, which lack peroxidase, will not be missed, and covers the testing necessary to subtype ALL. If CMS (e.g. Ia negative and/or T11 positive), blast morphology, or the clinical picture suggests T cell ALL, this is confirmed with T2 studies. E rosettes are not part of this basic protocol.

This protocol uses a range of commercial antisera readily available in New Zealand. As further antisera become accessible it may need to be modified particularly for typing myeloid leukaemia as some of the present myeloid antibodies appear to vary in positivity and strength of reaction.

Table 5 *Protocol for Cell Marker Testing in Acute Leukaemia*

Antibodies	Peroxidase Activity	
	Positive	Negative/Equivocal
Anti-TdT	X	X
Ia (I2)	X	X
MY4	X	X
MY7	X	
MY9	X	X
cALL (J5)		X
B1		X
T11		X
T2		(X)

Acknowledgement

The authors wish to acknowledge the contribution of Mary Ann White, Immunology Department, Auckland Hospital in performing additional immunological markers on some patients, in particular the confirmation of monoclonality in B ALL; Auckland Division of the Cancer Society of New Zealand (Inc) for providing funds for a cytocentrifuge; Lois Duncan for expert secretarial assistance; Lynne Logan for photography and art work.

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Human Leptospirosis in New Zealand

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Abstract

The history of studies on leptospirosis in New Zealand is presented. Studies both at the National Health Institute and at other centres are reviewed. The problems of laboratory diagnosis are discussed and commented upon.

Introduction

In April 1951, Kirschner and Gray[1] described an atypical spirochaetosis in a farmer suspected of having infectious hepatitis. He was found to have seroconverted to *Leptospira interrogans* serovar *icterohaemorrhagiae*. This identification of the first diagnosed human case of leptospirosis in New Zealand coincided with the establishment of reference facilities at the University of Otago. A year later Kirschner et al[2] serologically demonstrated the presence of serovar *pomona* in humans, calves and pigs. Pigs were implicated as the main carrier and the disease dubbed 'Swineherds Disease' after its European counterpart. Kirschner observed, however, that the clinical symptoms were more like the Australian *pomona* infections described in Queensland. It was further shown that leptospires survive long periods in tap water contaminated with milk. Control measures included the separation of cattle from the main carriers, pigs, and an assessment of antibiotic treatment was made.

In 1953 Faine and Kirschner[3] went on to show that the disease was widespread in New Zealand with an incidence of 0.35 per 10,000 population. Sixty-eight persons were serodiagnosed. It was noted that cases predominated in the North Island and that there the disease should be considered in cases of pyrexia of unknown origin (PUO).

West and Whitehead[4] described a case of serological conversion to serovar *canicola* in the Hutt Valley in 1953 and reported it as "canicola fever". It was assumed that the infection was zoonotic, originating from a dog.

Kirschner[5] published a review in 1954 of the work of the reference laboratory at the end of its second year of functioning. By this time the incidence had nearly doubled, and titres were demonstrated against the three serovars then known to be in New Zealand: *L. pomona, L. icterohaemorrhagiae* and *L. canicola*. Three patients were also found to have titres to the *hebdomadis* serogroup by using the *mitis* (Johnson) antigen newly introduced to the testing battery. The importance of serial serological investigations was stressed, especially if the stage of illness is unknown.

Leptospirosis diagnostic services were now transferred from Otago to the National Health Institute (NHI), and between 1954 and 1956 the service was consolidated and made comprehensive. Josland et al[6] at NHI validated Kirschner's earlier findings and went on to demonstrate wide cross-agglutinating reactions. In 1957 Kirschner and Maguire[7] produced a definitive article on the survival of leptospires outside their hosts. In this study dairy farmers were demonstrated to be the predominant occupational group affected and the term 'dairy farm fever' was introduced. The influence of bovine renal carriers, annual rainfall and mean atmospheric temperature on the persistence of leptospires in the rural environment and endemicity of the disease were discussed. Additionally, the study demonstrated the dramatic effect that rainfall had in changing milk, urine and fresh sewage from lethal environments for leptospires to surprisingly effective culture media.

During the next ten years antibiotic treatment changed the serological profile of leptospirosis. Therapy meant that lower titres developed and were observed later in the course of infection. Tennent and Philip in 1964[8] decried the continued usage of serology, and advocated a purely clinical diagnosis. Only the Department of Health and "compensation authorities", they argued, required serology. In 1966 Philip and Tennent[9] reported the usage of a leptospiral vaccine, containing *pomona*, on 362 susceptible people. Only seven failures were cited.

A multidisciplinary survey organised by the NHI in 1974[10] validated Kirschner's earlier work, but dissociated the pig from its previous theoretical role. Christmas et al[11] further commented on the low effectiveness of the vaccine. In this survey, discussed by Philip[12], the first New Zealand isolation of a member of the *hebdomadis* serogroup from blood cultures is documented. This was subsequently shown to be serovar *hardjo*. The serovar *hardjo* has been shifted from the *hebdomadis* to the *sejroe* serogroup. However, at that time it was in the *hebdomadis* serogroup. By then serovars *pomona* and *ballum* had also been isolated at the NHI (unpublished). In 1977 Thompson of the NHI isolated *L. interrogans* serovar *australis* from a 38-year-old dry-stock farmer in Dargaville[13].

Studies on the National Serum Bank (NSB) have shown that healthy blood donors in urban centres such as Auckland, Wellington and Christchurch[14] do not have significant antibody levels to the leptospiral serovars found in New Zealand. However in a further survey[15] which included some of the rural areas of the North Island a significant number of reactors, particularly to the *hebdomadis* serogroup, were found in the Taranaki and Waikato areas, but not in the Hawke's Bay area.

Recent Epidemiology

From 1981 to July 1983 there has been a steady overall decline in both the number of specimens received for testing at the NHI and in the number of confirmed cases of leptospiral infections. The year 1981 showed a peak leptospirosis season in the spring and early summer, with a sharp decline in March. However, in 1982-1983 the peak leptospirosis season shifted to the late summer and early autumn months of February, March and April, with a decline in May. The number of confirmed cases in the leptospirosis season of 1982-1983 was greatly reduced from 40 cases in March 1982 to 17 cases at the same time in 1983.

In the period under study a *hardjo/pomona* vaccine for cattle was made available. This vaccination was designed to protect the farmer as opposed to the earlier *pomona* vaccine which attempted to protect animals. *Hebdomadis* infection in cattle is often sub-clinical, therefore the farmer may not be aware of it.

The predominant infecting serogroups continued to be *hebdomadis* and *pomona*, although serological evidence of serovars *tarassovi* and *ballum* continued to be found as well. Cross-reactions between serovars *pomona* and *bratislava* caused some serological confusion over the identity of the infecting serogroup as serovar *bratislava* has never been isolated in New Zealand. In some cases a titre to serovar *bratislava* precedes a higher titre to serovar *pomona*, which only becomes evident two to three weeks later. Other examples of reactions recently observed are given in Table 1.

As to be expected, the geographical distribution of leptospirosis is mainly confined to the endemic districts of New Zealand: Whangarei, Taranaki, Hauraki Plains.

Discussion

Following on from Kirschner's earlier observations[5,7] on the effect of weather and temperature on the endemicity and periodicity of leptospirosis, it could well be that the reduction in

Table 1
Bratislava/Pomona Cross Reactions as Observed in Three Different Patients

Patient	Date Specimen Taken	Reciprocol titres to serovars: *Bratislava*	*Pomona*
A	13.4.83	400	100
	18.4.83	400	200
	9.5.83	400	400
B	19.6.82	3200	<50
	27.6.82	3200	100
	8.7.82	3200	100
C	8.10.82	<50	5120
	27.10.82	<50	2560

incidence of the disease since spring and summer of 1982 is directly related to the near-drought conditions in many of the endemic areas in 1983. It also follows that an accurate assessment of the new cattle vaccine will have to wait until more appropriate climatic conditions prevail.

Because of the continuing heavy reliance on serology and despite the relative ease of cultivation of leptospires, there are still some anomalies. The most interesting, and potentially most confusing, observation is that a rising titre to serovar *bratislava* often, but not always, precedes seroconversion to serovar *pomona*. In addition seroconversion to serovar *tarassovi* occurs from time to time mainly in specimens received from Whangarei and Wanganui. Conversely, despite the isolation of serovar *australis* in 1977, no significant serological evidence has been found for this serovar in the general population. In this particular case seroconversion was only very weak, and it should be noted that similar low titres are often observed during infections with serovar *ballum*. These facts further stress the importance of isolation and cultivation for the effective diagnosis of leptospirosis, despite the unduly heavy reliance on serological techniques.

Acknowledgement

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High-resolution Chromosome Banding

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Abstract

Method for the production of elongated human chromosomes from blood, and fibroblast cultures for visualization of high-resolution Giemsa-trypsin banding are discussed, alongside their application in the busy routine clinical cytogenetic laboratory.

Key Words

Deletion syndromes; High resolution trypsin Giemsa-banding; Human chromosomes.

Introduction

Using G-, C-, R-, and Q-banding techniques metaphase chromosomes have been used exclusively in routine chromosome analysis and to a large degree this continues to be the present position. The importance of high-resolution banding in clinical diagnoses and the need for improved banding resolution to aid accuracy in gene mapping has long been recognised.[1,2] Observations by different workers[3,4] have shown that band numbers could reach at least 450 per haploid set when chromosomes were analysed in the longer prophasic state compared with 320-330 bands per haploid genome at metaphase. Using controlled culture harvest times with minimal strength colcemid to obtain peak mitotic index (thus more early metaphases and late prophases) Yunis and Sanchez[5] were able to assess up to 1,000 bands per haploid set.

Since 1975 the development of many cell synchronization techniques using thymidine,[6,7] amethopterin,[8-10] and bromodeoxyuridine[11] to obtain consistently high percentages of well spread, good quality early mitotic chromosomes has resulted in about 1,000 bands per haploid set of chromosomes being easily achieved. Even higher numbers of bands were obtained by Yunis[12] when he combined cell synchronization with the addition of bromodeoxyuridine (BrdU) and actinomycin D (AMD) to cell cultures resulting in 1700-2000 bands per haploid genome.

It is on the background of this research that techniques now used in clinical cytogenetics are based and subsequently modified to suit the individual circumstances of a particular laboratory. It is the aim of this paper to highlight those methods which the author's laboratory have found to be most successful and to outline in brief other existing ones.

Materials and methods

Methods for obtaining mid-prophase human chromosomes from lymphocytes

METHOD 1. Modification of Yunis[12]

Solutions and Reagents.

1. Amethopterin (Methotrexate; Lederle Laboratories division).
2. Actinomycin D (Dactinomycin; Merck, Sharp and Dohme).
3. Bromodeoxyuridine (BrdU; Sigma).
4. Colcemid (Gibco).

Procedure

1. Chromosomes are set up in the normal way (0.5mL of

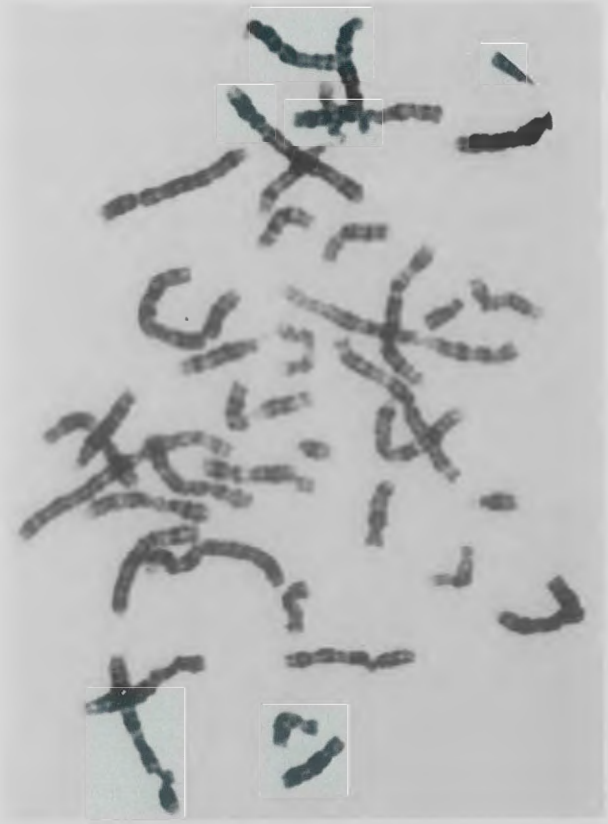

Fig. 1. *Early metaphase cell showing chromosomal damage from actinomycin D.*

heparinised whole blood in 10.0 mL of tissue culture medium at 37°C.)

2. After 65-72 hours amethopterin is added to the cultures at a final concentration of 10^{-7} M (i.e., 100μL of 10^{-5} M solution/ 10mL culture).
3. The cultures are reincubated for a further 17-18 hours at 37°C.
4. At the end of this time the lymphocytes are released from the block by washing cells x2 in warm medium (37°C) and resuspending in 10.0 mL of fresh tissue culture medium enriched with 12μg/mL of BrdU.
5. Cultures are reincubated for a further 5 ½ hours at 37°C.
6. At the end of this time actinomycin D is added to the cultures at a concentration of 5.0μg/mL and the cultures reincubated for a further 45 minutes at 37°C.
7. At the end of 45 minutes colcemid (0.5μg/mL) is added to the cultures for 12-15 minutes and reincubated at 37°C.
8. Chromosomes are harvested in the normal way except that they are resuspended in hypotonic solution (0.075 M KCl) for 15 minutes at 37°C.

Fig. 2. *Typical mid-prophase cell showing high- resolution trypsin G-banding with inevitable overlaps.*

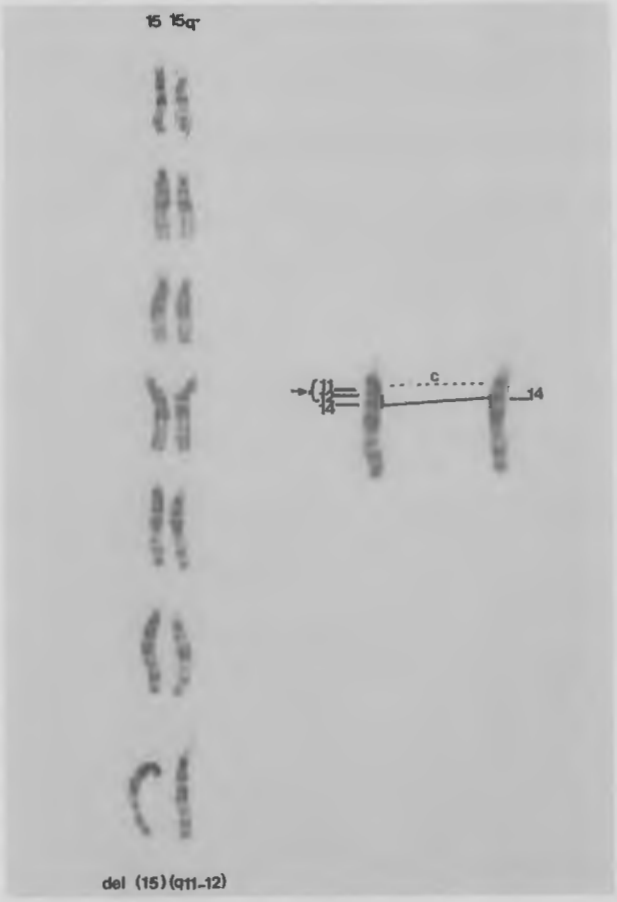

Fig. 3. *Case of Prader-Willi syndrome showing deletion of bands 15q 11-12.*

Fig. 4. *Normal chromosome 13 showing breakpoint site in Retinoblastoma.*

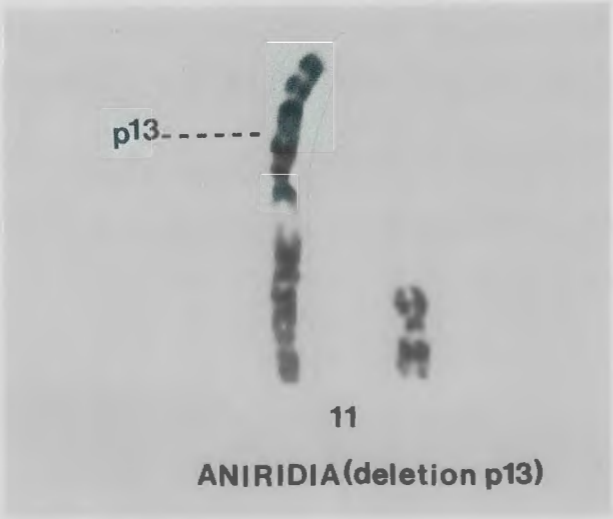

Fig. 5. *Normal chromosome 11 showing breakpoint site in Aniridia.*

9. Cells are fixed in 3:1 methanol/acetic acid. After initial fixation for 20 minutes fixative is changed x4.
10. Spreading is achieved by dropping appropriate cell suspension onto clean slides from a height of approximately 3 feet.
11. High-resolution trypsin G-bands are then obtained by treating 3-5 day old slides in weak trypsin solution 0.25 mL of BACTO trypsin diluted to 20.0 mL with pH 6.8 Sorensen's phosphate buffer.
12. Slides then rinsed in pH 6.8 phosphate buffer stained with Leishman stain for 4 minutes, rinsed in pH 6.8 buffer and blotted dry.

Notes on Procedure

1. Amethopterin (methotrexate) inhibits the enzyme dihydrofolate reductase thereby preventing the formation of thymidine monophosphate from deoxyuridine monophosphate and blocking DNA replication. Cells are prevented from traversing the cell cycle and accumulate at the G_1/S border. After release of the block, cells complete DNA replication and may be arrested in synchrony.
2. When cells are exposed to actinomycin D during the G_2 period of cell cycle the normal process of chromosomal condensation prior to and during mitosis is partially inhibited resulting in elongated chromosomes. The elongation effect is more pronounced in the G-negative bands since they are believed to be enriched in guanine-cytosine base pairs. If used in concentrations of over 6.0μg/mL actinomycin D can

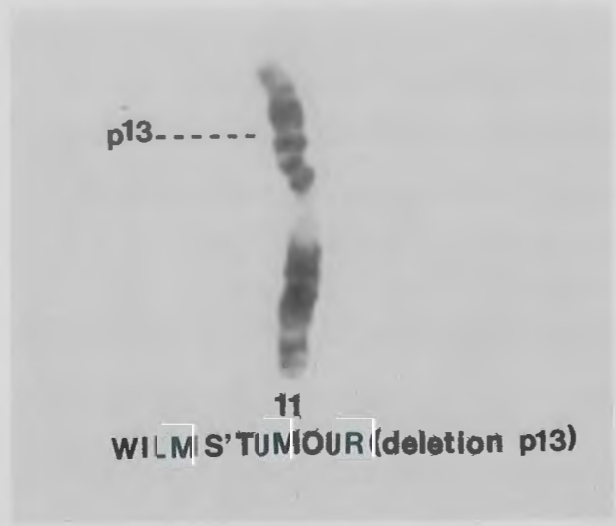

Fig. 6. *Normal chromosome 11 showing breakpoint site in Wilms' Tumour.*

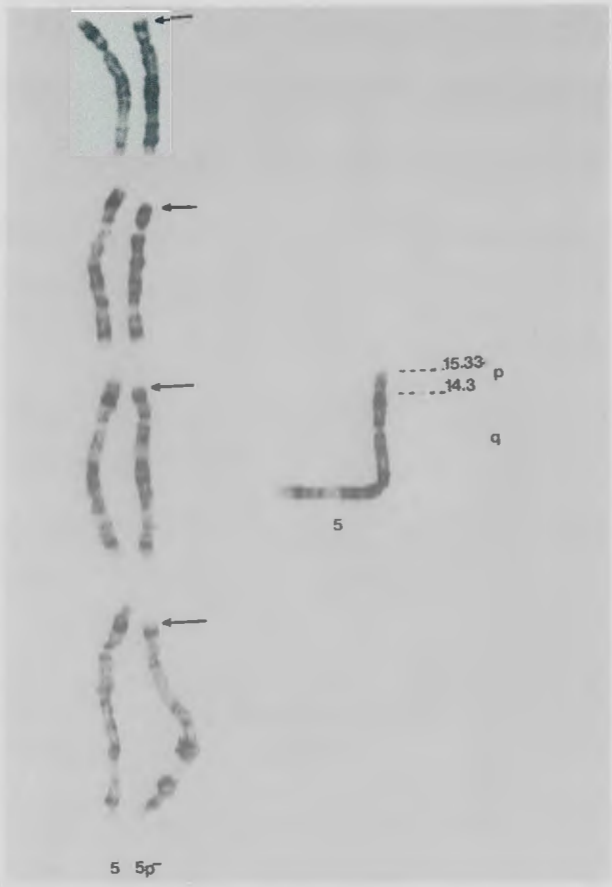

Fig. 7. *Case of Cri-du-Chat showing deletion 5q 15.32-14.3.*

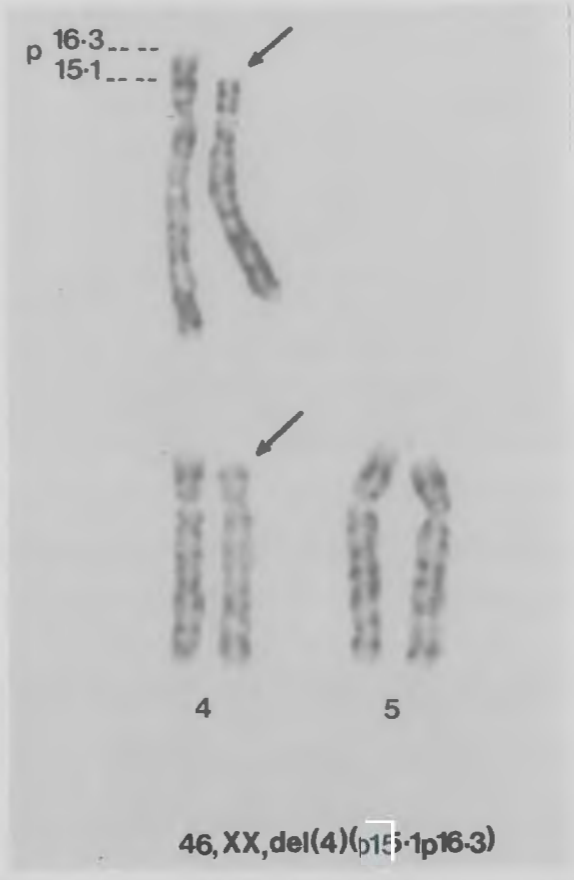

Fig. 8.(a) *Case of Wolf-Hirschhorn syndrome showing deletion 4p 15.1-16.3.*

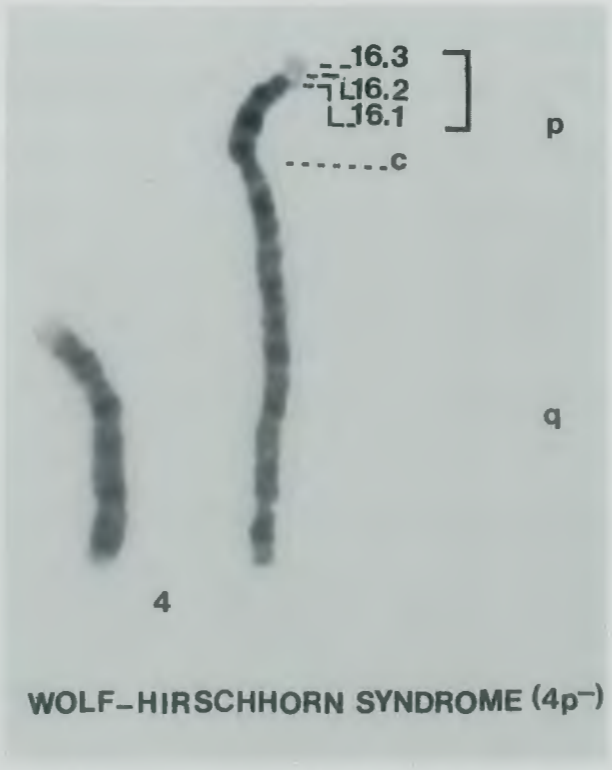

Fig. 8.(b) *Normal chromosome 4 showing bands involved in deletion to present with Wolf-Hirschhorn syndrome.*

adversely effect mitotic index and cause unacceptable chromosomal damage (Fig. 1).

3. Bromodeoxyuridine (BrdU) is another agent that produces chromosome elongation and improves definition of chromosomal bands. Disadvantages of BrdU are that it depresses mitotic index and causes chromosomal breakage. In addition, cells show a differential elongation effect which may vary from chromosomal region to chromosomal region.
4. Even with good spreading techniques, overlapping of chromosomes will occur in early mitotic cells. However as can be seen in figure 2 the wealth of band information is great and by further analysis of other cells the whole genome may be successfully screened.
5. The modification of this method by Yunis[12] is in our estimation very reliable. It does mean however that as the culture period covers a time span of 96 hours, specimens may only be set up on Mondays or Fridays in a normal working week. Likewise cultures need to be incubated late in the evening 2200- 2300 hours to accommodate the procedure after cells have been released from block, so that mitotic peaks may be met. Nevertheless for important cases specimens can be organised to meet these demands.

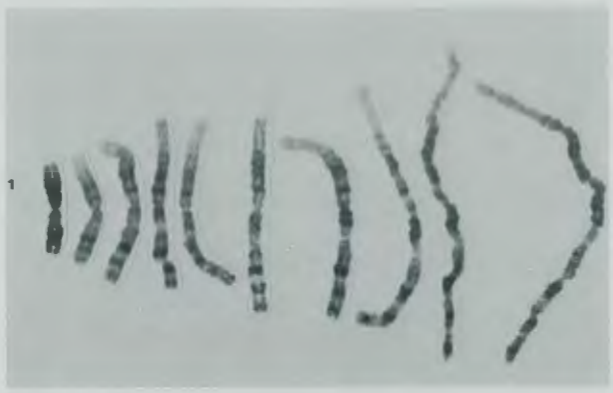

Fig. 9. *Chromosome 1 from metaphase to mid- prophase.*

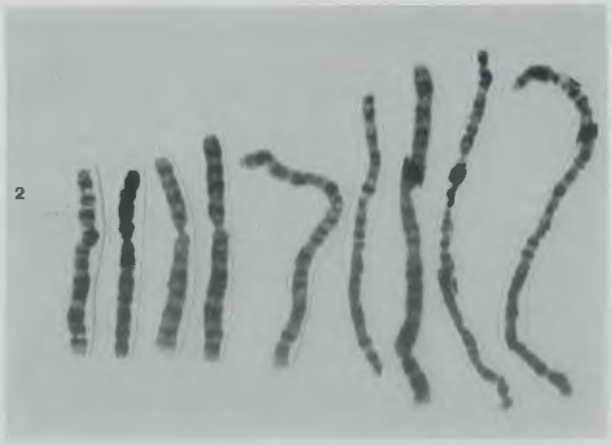

Fig.10. *Chromosome 2 from metaphase to mid- prophase.*

METHOD 2. Modification of Rybak et al[13]

Solutions and Reagents: As for Method 1.

Procedure:

1. Human blood cultures are set up in the normal way.
2. After 64 hours incubation at 37°C cultures are treated with actinomycin D (5.0μg/mL) and colcemid (0.5μg/mL) and reincubated for a further hour.
3. Cells are harvested in the normal way except that they are resuspended in hypotonic solution (0.075M KCl) for 15 minutes.
4. Cells are fixed in 3:1 methanol/acetic acid. After initial fixation for 20 minutes fixative is changed x4.
5. High-resolution trypsin G-bands are performed as in Method 1.

Notes on Procedure:

1. Mitotic peaks vary and therefore the final hour of incubation may not be at 64 hours but 65-68 hours. One may have to experiment away from the rigidity of the method to suit laboratory conditions.
2. In their paper Rybak et al[13] also gave an alternative method where BrdU was added to the cultures after 58 hours of incubation and then the same procedure was followed. In their method they use BrdU at a concentration of 210μg/mL. Our experience shows this to greatly reduce mitotic activity and suggests that 50-100μg/mL is more appropriate.
3. The methods of Rybak et al[13] have the advantage of being able to be performed on 72 hour cultures. In our estimation the techniques do not produce as many early mitotic figures as does Yunis's[12] technique but nevertheless the methods are shorter and less involved.

One stage methods for the accumulation of early mitotic cells in human lymphocyte cultures

Without going into vast detail because reference will be given, the

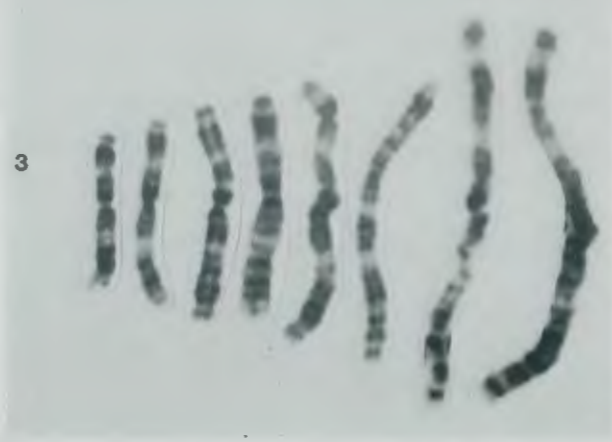

Fig.11. *Chromosome 3 from metaphase to mid- prophase.*

following methods are being used with more frequency and success.

METHOD 1. USE OF ETHIDIUM BROMIDE (Ikeuchi and Sasaki[14]; Ikeuchi[15])

Procedure:
Human lymphocytes are cultured for 64-72 hours in the normal way and exposed to ethidium bromide (5-10.0μg/mL) and colcemid (0.2μg/mL) simultaneously for 1-2 hours then routinely harvested and G-banded as for proceeding methods.

METHOD 2. USE OF ACRIDINE ORANGE (Matsubara and Nakagome[16])

Procedure:
Human chromosomes are grown for 72-96 hours in the normal way. Colcemid (0.025-0.03μg/mL is added for 1 ½ hours. During the final 30 minutes acridine orange (20-25 μg/mL) is added to the culture. Hypotonic treatment using 0.75 M KCl is carried out for 25-30 minutes at 37°C. Banding is performed as for proceeding methods.

Methods for high-resolution bands in human fibroblasts and amniotic fluid cells

METHOD 1. Modification of Yu et al[17]

Procedure:
1. Skin fibroblasts are prepared in the normal way.
2. 24-48 hours after sub-culturing (when appropriate numbers of cells are "rounding up") colchicine or colcemid is added for 4-6 hours.
3. 1 hour prior to harvest actinomycin D (2.0μg/mL) is added to the culture.
4. Cells are harvested and spread in the normal way.
5. Banding is performed on slides 3-5 days old.

METHOD 2. Hoo et al[18]

Procedure:
Ethidium bromide 5μg/mL is added to actively growing and dividing monolayers of amniotic fluid cells for 4 ½ hours. Colcemid 0.05μg/mL is added for the last 1 ½ hours.

Harvesting and banding is carried out in the normal way.

METHOD 3. Jager and Kuhn-Schlage[19]

Procedure:
Two days after trypsinization amniotic fluid cells were incubated for 4 hours in culture medium containing 20U/mL liquemin. During the last hour 5μg/mL ethidium bromide is added and 15 minutes before harvest colcemid 0.04 g/mL is applied. Harvesting and banding is performed in the normal way.

Notes on procedure:
1. In our experience we have found that for amniotic fluid cells and skin fibroblast cultures there is generally (if properly read before the addition of colchicine or colcemid) enough mitotic activity to produce cells in early division without resorting to the use of elongation agents such as actinomycin D, ethidium bromide or BrdU.

Discussion

Current standard banding techniques detect an abnormality in some 10-15% of all patients referred for cytogenetic studies[9]. With increased usage of high-resolution banding this figure would be expected to rise. Already this has been evident in patients with Prader-Willi syndrome, Retinoblastoma, Aniridia, Wilms' Tumour, Wilms'-Tumour-Aniridia syndrome and Aniridia-Geneto-urinary mental Retardation syndrome. Since 1976 chromosomal rearrangements involving chromosome 15 have been observed in Prader-Willi syndrome[20-25]. High resolution banding has made it possible to demonstrate that band 15q 11-12 has been involved (Fig 3). The loss of this segment is estimated to be in at least half of the patients presenting with this condition. Likewise in Retinoblastoma[26-32] deletion of 13q14 has been found (Fig 4) also isochromosome of 6p and trisomy 1q have been identified from Retinoblastoma tumours[30,32]. In Aniridia (Fig 5) and Wilms' Tumour (Fig 6) and its' associated syndromes deletion 11p13 has been demonstrated[33-35].

In other well established syndromes such as Cri-du-Chat (Fig 7) Wolf-Hirschhorn (Fig 8. a,b) the critical regions have been assessed as 5p 15.2 and 4p 16.1.-16.3 respectively. Re-analysis

Fig.12. *Chromosomes 4, 5 and 6 metaphase to late prophase.*

of phenotypically abnormal patients with apparent balanced structural rearrangements using high-resolution banding have shown no definite imbalance confirming translocation reciprocity and in some cases breakpoints were reassigned to sub-bands and others to totally different bands[36]. Ring chromosomes too have come in for close scrutinization showing in some instances no detectable loss of chromatin[37].

The application of elongated chromosome banding techniques in the study of bone marrow cells from patients with pre-leukaemic and leukaemic conditions has resulted also in the identification of specific chromosome rearrangements of diagnostic and prognostic value. I have purposefully refrained from highlighting any specific methodology as being superior for the production of elongated chromosomes in leukaemia as the available techniques are based on existing methods for blood cultures. Personal experience has shown great variability in their

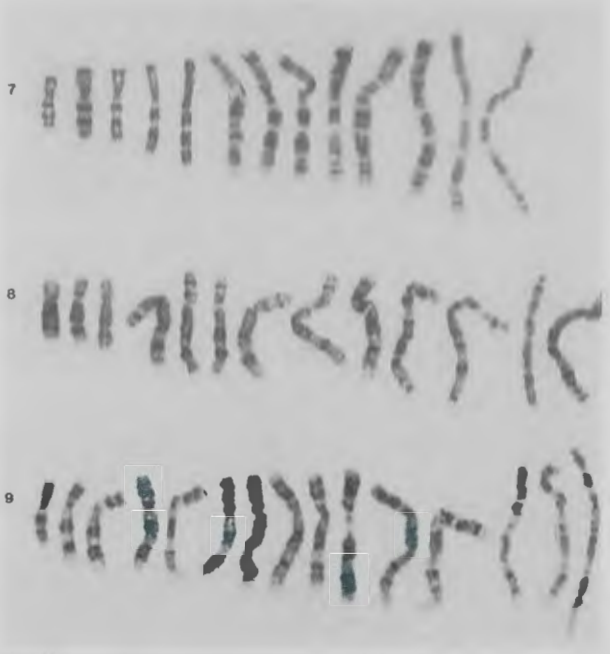

Fig.13. *Chromosomes 7, 8 and 9 metaphase to mid-prophase.*

reproduction which is not totally unexpected considering the range in quality of specimen and spectrum of haematopoietic disorders. Nevertheless, there is a real need for those engaged in this specialty to adopt a method which is reasonably consistent for everyday analysis. An excellent article discussing the problem was written by Gallo et al[38].

That the high-resolution techniques are more complex and time consuming than routine procedures is not in dispute, so that it would be impossible to apply them to every peripheral blood study. When, however, syndromes which are recognised as having small deletions are being investigated then these methods are essential in aiding accurate diagnosis[39]. Although concurring that it is not possible to subject every sample to the procedures, in the author's laboratory we have found that normal culturing and harvesting of 96 hour TC199 cultures produce without any pre-treatment a significant number of early mitotic divisions for examination for duplication/deficiency of chromatin.

I have discussed at length the virtues of what the cells in early division are able to accomplish in diagnostic value but what of their morphology? Chromosomes go through an extreme process of contraction from their highly dispersed interphase appearance to the highly contracted appearance of late metaphase, thus the number of bands perceived along the length of a chromosome is not constant but is dependent on the stage of contraction of that particular cell. The high-resolution studies have shown that each metaphase band can comprise three to five prophasic bands as illustrated in Figs. 9-17. Since such large numbers of bands exist in early metaphase and prophase a new identification nomenclature has been devised (ISCN 1981)[40] and thus the cytogeneticist has had to come to terms with a whole new range of individual chromosome "patterns".

I have previously made mention of the more well-known established deletion syndromes but recently the Di George syndrome[41,42] has been associated with deletion 22q 11, the Langer-Giedion syndrome[43] with deletion 8q 23 and the Miller-Dieker syndrome[44] with deletion 17p13. Nevertheless chromosome analysis even with prophasic type chromosomes is severely limited by the resolving power of the microscope. Assuming that there are maximally 100 000 genes in man and that the maximum number of bands definable is 2 000, then the smallest band that can be detected with the eye-light-microscope combination will contain about 50 genes. Ultimate resolution of chromosomes at the gene level will eventually be possible by the techniques of recombinant DNA technology, but until that era high-resolution chromosome banding is a very adequate means of diagnostic technology in medicine.

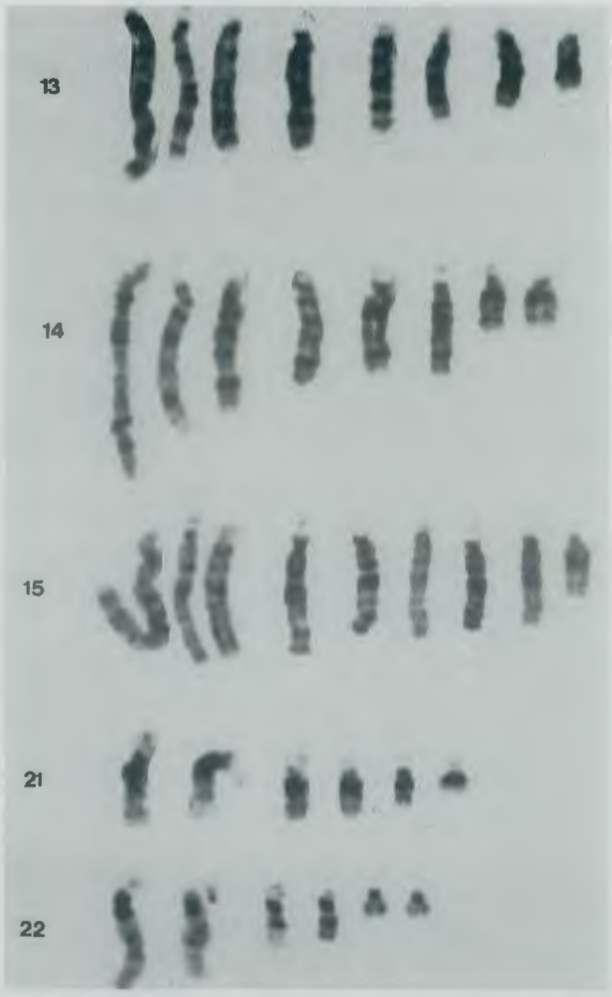

Fig.15. *Acrocentric chromosomes metaphase to late-prophase.*

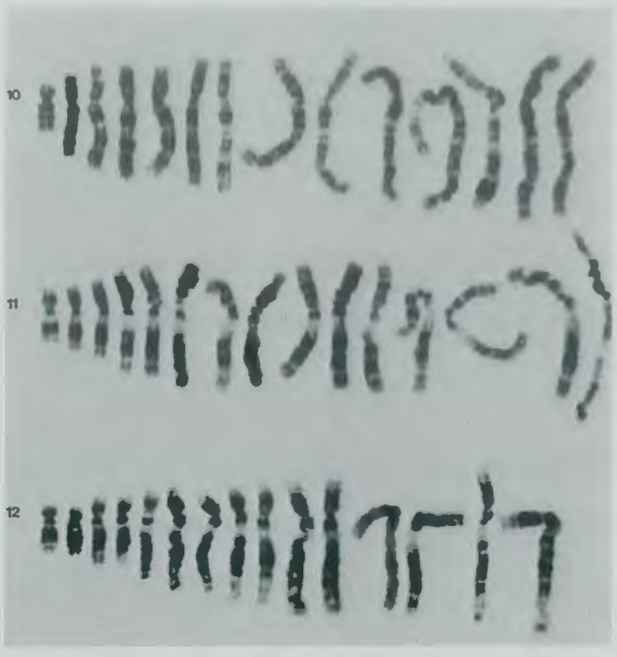

Fig.14. *Chromosomes 10, 11 and 12 metaphase to mid-prophase.*

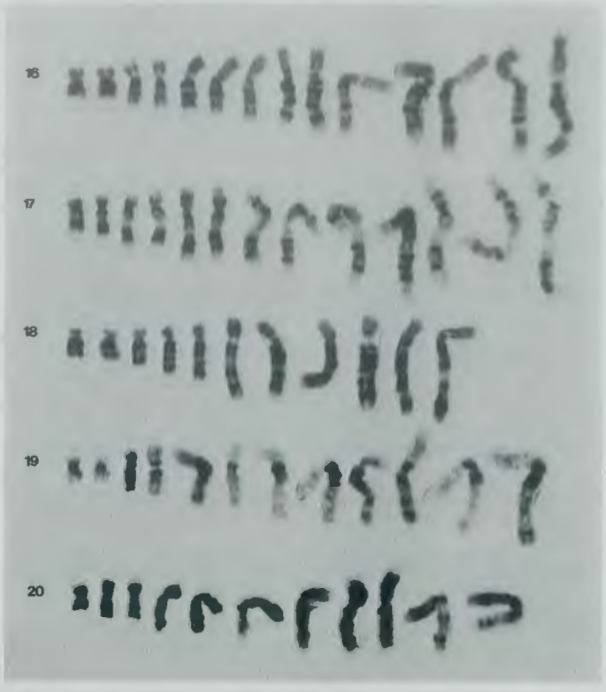

Fig.16. *Chromosomes 16, 17, 18, 19 and 20 metaphase to mid-prophase.*

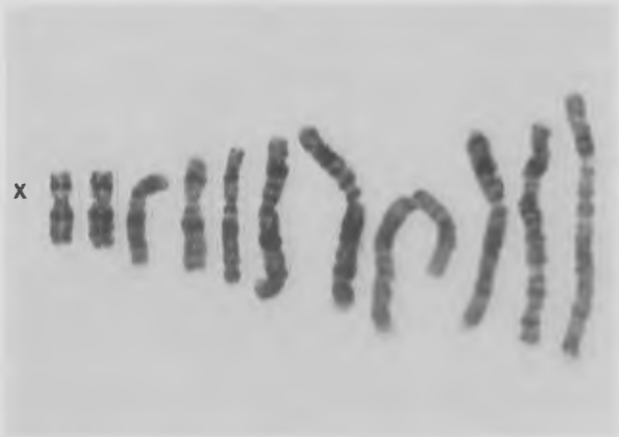

Fig.17. *X Chromosome metaphase to mid-prophase.*

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Technical Communication

Fibrin Degradation Products. An Evaluation of a Newly Available Commercial Latex Reagent.

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Introduction

The degradation of fibrin/fibrinogen by plasmin results in the formation of fragments of varying molecular size known collectively as fibrin/fibrinogen degradation products (FDPs). There are four major degradation products X, Y, D and E fragments. When crosslinked fibrin is degraded, crosslinked fragments including D-dimer are produced.

Several methods are available for the detection of FDP's in serum and these include such assays as tanned red cell haemagglutination inhibition immunoassay, staphylococcal clumping test and the now more commonly used assays using latex particles coated with specific antisera.

We have recently evaluated three different commercial latex reagents in our laboratory. The reagents used were Thrombo-Wellcotest (Wellcome Laboratories) which consists of latex particles coated with antibodies to fragments D and E, Dimertest-Latex (MAbCo) in which latex particles are coated with a monoclonal antibody D.D.3B6/22 specific for D-dimer, and FDP latex reagent (Mercia-Brocades Ltd) a newly available reagent which consists of latex particles coated with antibody to fragment E. Thrombo-Wellcotest and Dimertest are routinely used in our laboratory.

Methods

1. All three methods were performed essentially as described in the manufacturers instructions. Samples which were positive at a 1:5 dilution were investigated further using serial dilutions of serum from 1:10 to 1:100.
2. Blood samples were collected into glass test tubes containing Trasylol at a concentration of 250 KIU units per millilitre of whole blood, and incubated at 37°C for at least four hours to ensure complete clotting. Samples giving positive results were separated and retested following further incubation.

Results

Over a period of five days eleven samples on ten patients were assayed with each of the three reagents.

Six specimens had FDP levels within the normal range of up to 10mg/L using Thrombo-Wellcotest and Mercia-Brocades reagents. Three of these samples had mildly increased levels of D-dimer at 0.2-1mg/L. Four specimens had slightly increased levels of FDP with 20-80 mg/L using Thrombo-Wellcotest. The results using Mercia-Brocades reagent were either the same or positive at one dilution lower. D-dimer levels on these samples were between 1.0 and greater than 2.0 mg/L.

In one patient with frank Disseminated Intravascular Coagulation (DIC), TFDP levels using both Thrombo-Wellcotest and Mercia-Brocades reagents were greater than 200 mg/L and greater than 2.0 mg/L for D-dimer. However the results on the initial dilutions of 1:5 to 1:30 using the Mercia-Brocades reagent were negative, with the degree of positivity increasing with dilution up to 1:100.

Because of this apparent prozone effect we thawed samples of serum stored at -30°C from other patients known to have greatly increased levels of FDP. All samples had other laboratory evidence of DIC. FDP levels on eleven samples were estimated using the three reagents. All samples had Thrombo- Wellcotest FDP levels of greater than 200 mg/L and D-dimer levels of greater than 2.0 mg/L. FDP levels using Mercia-Brocades reagent ranged from 120 mg/L to greater than 200 mg/L. The prozone effect was noted in 10 samples.

Discussion

If the manufacturers instructions as supplied with the Mercia-Brocades reagent were carried out and samples were screened using dilutions of 1:5 and 1:20, eight of the ten samples would have had a positive result by the 1:20 dilution. False negative results were therefore seen in two samples which, with further dilution, were shown to have FDP levels of greater than the 200 mg/L.

Copies of our results were sent to the manufacturers of the reagent by the New Zealand distributors Med Bio Enterprises Ltd for their comment. It seems that Mercia-Brocades have recently become aware of this problem and now include further instructions suggesting that if DIC is suspected on other grounds then the test should be repeated using a dilution of 1:200.

Med Bio Enterprises, acting on our advice have recommended to New Zealand users of this reagent that dilutions of 1:5 and 1:50 be used to screen samples for FDP as we felt these to be more appropriate dilutions. We wonder if in some cases a 1:200 dilution may in fact be too high.

We are concerned that for economic reasons laboratories may use only a 1:5 dilution of serum as a screening test believing that if this is negative the results are normal and therefore fail to obtain a very high FDP result which would aid in the laboratory diagnosis of DIC.

We would recommend that laboratories using this reagent use at least 1:5 and 1:50 dilutions of serum to screen for the presence of FDPs. If DIC is suspected on other laboratory evidence, then further dilutions may need to be carried out.

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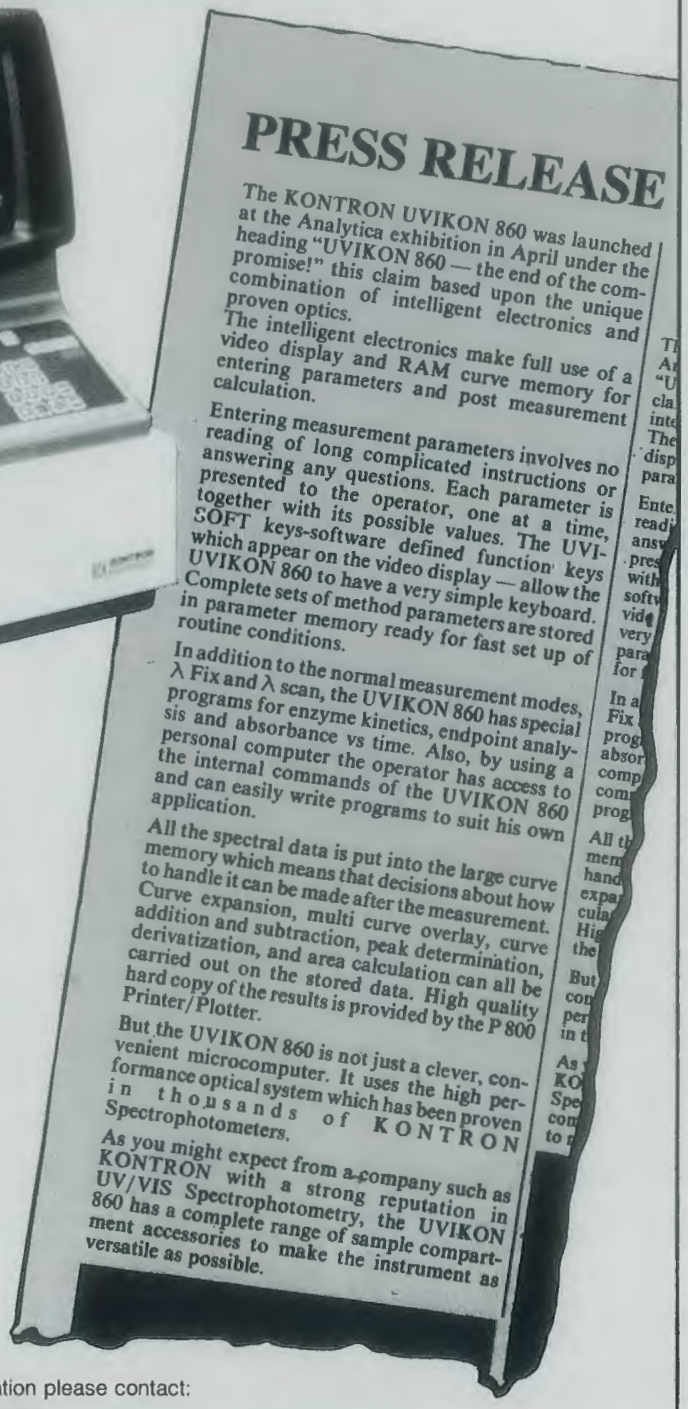

PRESS RELEASE

The KONTRON UVIKON 860 was launched at the Analytica exhibition in April under the heading "UVIKON 860 — the end of the compromise!" this claim based upon the unique combination of intelligent electronics and proven optics.

The intelligent electronics make full use of a video display and RAM curve memory for entering parameters and post measurement calculation.

Entering measurement parameters involves no reading of long complicated instructions or answering any questions. Each parameter is presented to the operator, one at a time, together with its possible values. The UVI-SOFT keys-software defined function keys which appear on the video display — allow the UVIKON 860 to have a very simple keyboard. Complete sets of method parameters are stored in parameter memory ready for fast set up of routine conditions.

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— Rather than list all specifications we would urge those interested in spectrophotometers to read the following press release.

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Continuing Education

Revision Series in Biochemical Calculations
Section V: pH Calculations Part C — Buffer Solutions

Trevor A. Walmsley and Michael Lever
Dept. of Clinical Biochemistry, Christchurch Hospital, Christchurch.

Buffer Solutions

The buffer solutions we meet in the laboratory (or simply "buffers") are solutions which resist a change in pH on the addition of small quantities of acid, base or solvent. A buffer solution contains a weak acid or base and its corresponding salt.

Consider the dissociation of a weak acid HA (see Section IV)

$$HA \rightleftarrows H^+ + A^-$$

Acid Salt

The acid dissociation constant K_a is given by:—

$$K_a = \frac{[H^+][A^-]}{[HA]}$$

Rearranging:—

$$[H^+] = K_a \times \frac{[HA]}{[A^-]}$$

taking logs:—

$$-\log [H^+] = -\log K_a - \log \frac{[HA]}{[A^-]}$$

$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

In general $$pH = pK_a + \log \frac{[salt]}{[acid]}$$

where [acid] = concentration undissociated acid
[salt] = concentration of salt of its corresponding weak acid

This is the "Henderson-Hasselbalch" equation — as can be seen from its derivation above it is an **alternative** way of expressing the dissociation constant.

Also it can be shown that:

$$pOH = pK_b + \log \frac{[salt]}{[base]}$$

However this is not widely used and most tables of dissociation constants don't give the pK_b for weak bases but list the acid dissociation constants of the salts of weak bases.

For example the acid dissociation of $R.NH_3^+$ as in organic amines or amino acids is given by:—

$$R.NH_3^+ \rightleftarrows H^+ + R.NH_2$$

Acid Salt

therefore $$pH = pK_a + \log \frac{[R.NH_2]}{[R.NH_3^+]}$$

where

$[R.NH_2]$ = [salt] = concentration of amine
$[R.NH_3^+]$ = [acid] = concentration of amine salt

Note that in the acid dissociation of salts of weak bases the "acid" is the salt of the weak base and the "salt" is the base.

Buffer Resistance

Buffer Resistance is a measure of the resistance of a buffer to pH change and is given by the rate of change of pH with respect to the rate of addition of hydrogen ions i.e.

$$\text{Buffer Resistance} = \frac{\Delta[H^+]}{\Delta pH}$$

The most efficient buffer system occurs when there is equal concentration of acid and its corresponding salt i.e. [acid] = [salt].

since $$pH = pK_a + \log \frac{[salt]}{[acid]}$$

therefore $$pH = pK_a + \log 1$$

$$pH = pK_a$$

Therefore the most efficient buffer system occurs when a buffer has a pH equal to the pK_a. As a practical "rule of thumb" buffer resistance is adequate if:—

$$\frac{1}{10} < \frac{[salt]}{[acid]} < \frac{10}{1}$$

i.e. $$pH = pK_a \pm 1$$

Hence the practical useful buffer range for an acetic acid/acetate buffer (pK_a = 4.76) would be about 3.8 - 4.8 and the buffer range for an ammonia/ammonium buffer (pk_a =9.24) would be about 8.2 - 10.2. Thus different buffer systems must be selected to prepare buffers covering different parts of the pH scale.

Examples of Calculating the pH of Buffer Solutions

Example 1 *Calculate the pH of a solution containing 0.05 mol/L acetic acid and 0.025 mol/L sodium acetate. Given pK_a = 4.76*

To solve the problem we need to calculate the concentration of salt (acetate ions) and acid (acetic acid).

The acetic acid is only partially dissociated

$$HAc \rightleftarrows H^+ Ac^-$$

thus the concentration of acid [HAc] = 0.05 - $[H^+]$ mol/L

however $[H^+]$ is very small in relation to [HAc]

therefore [HAc] = [0.05] mol/L approximately

The sodium acetate being a salt is completely dissociated occurring always as Na^+ and Ac^-.

therefore $[Ac^-] = 0.025$ mol/L

$$pH = pK_a + \log \frac{[salt]}{[acid]}$$

$$= 4.76 + \log \frac{0.025}{0.050}$$

$$= 4.46$$

Example 2 *Calculate the pH of a solution prepared by mixing 100 ml of 0.1 mol/L acetic acid with 25 ml of 0.1 mol/L sodium hydroxide.*

However 1 mole of sodium hydroxide (NaOH) reacts with 1 mole of acetic acid (HAc):—

$$(Na^+) + OH^- + HAc \rightarrow H_2O + Ac^- + (Na)$$

Note the sodium ions don't take part in the reaction but are included to stress that the solution remains electrically neutral throughout all stages of reaction.

$$\text{number of moles HAc} = \frac{100 \times 0.01}{1000}$$

$$= 0.01 \text{ moles}$$

$$\text{number moles NaOH} = \frac{25 \times 0.1}{1000}$$

$$= 0.0025 \text{ moles}$$

Therefore 0.01 moles HAc is mixed with 0.0025 moles of NaOH to give a solution containing 0.0025 moles Na^+ ions, 0.0025 moles Ac^- ions and leaving 0.0075 moles of unreacted HAc.

therefore $$pH = pK_a + \log \frac{[Ac^-]}{HAc}$$

$$= 4.76 + \log \frac{0.0025}{0.0075}$$

$$= 4.28$$

Example 3 *How would you prepare a 0.1 mol/L acetate buffer pH 4.40 from 1 mol/L acetic acid solution and 1 mol/L sodium acetate? What volume of 1 mol/L sodium hydroxide should be added to 1 litre of buffer to change its pH from 4.40 to 5.00?*

$$pH = 4.76 + \log \frac{[Ac^-]}{[HAc]}$$

therefore at pH 4.40

$$\log \frac{[Ac^-]}{[HAc]} = -0.36$$

taking antilogs

$$\frac{[Ac^-]}{[HAc]} = 0.436$$

$$[Ac^-] = 0.436 \times [HAc] \quad \text{..... (3-I)}$$

However since we are preparing a 0.1 mol/L acetate buffer

$$[HAc] + [Ac^-] = 0.1 \text{ mol/L}$$

therefore $$[Ac^-] = 0.1 - [HAc] \text{ mol/L}$$

substituting in Eq 3-I

$$0.1 - [HAc] = 0.436 \times [HAc] \text{ mol/L}$$

$$0.1 = 1.436 \times [HAc] \text{ mol/L}$$

therefore $$[HAc] = 0.070 \text{ mol/L}$$

substituting in Eq 3-I

$$[Ac^-] = 0.030 \text{ mol/L}$$

Therefore to prepare 1 litre of 0.1 mol/L acetate buffer we require 70 mL of 1 mol/L acetic acid and 30 mL of 1 mol/L sodium acetate.

If we now add sodium hydroxide to this solution the pH will increase and at pH 5.00 the log ratio of acetate to acetic acid is given by:—

$$\log \frac{[Ac^-]}{[HAc]} = 5.00 - 4.76$$

$$= 0.24$$

taking antilogs

$$\frac{[Ac^-]}{[HAc]} = 1.738$$

therefore $$[Ac^-] = 1.738 \times [HAc] \quad \text{..... (3-II)}$$

But since this buffer is still a 0.1 mol/L acetate buffer

$$[HAc] + [Ac^-] = 0.1 \text{ mol/L}$$

therefore $$[Ac^-] = 0.1 - [HAc] \text{ mol/L}$$

substituting in Eq 3-II

$$0.1 - [HAc] = 1.738 \times [HAc]$$

$$0.1 = 2.738 \times [HAc]$$

therefore $$[HAc] = 0.037 \text{ mol/L}$$

substituting in Eq 3-II

$$[Ac^-] = 0.063 \text{ mol/L}$$

Therefore we need to convert 0.070 mol/L acetic acid at pH 4.40 to 0.037 mol/L acetic acid at pH 5.00 — to achieve this we require 0.033 mol/L of sodium hydroxide (0.070 - 0.037). Therefore we need to add 33 mL of 1 mol/L sodium hydroxide to change the pH from 4.40 to 5.00.

Solution Equilibria

In order to solve pH problems in solutions of polybasic electrolytes it is necessary to understand the reactions occurring in that solution.

For example if we mix equal volumes of 0.2 mol/L tartaric acid and 0.2 mol/L sodium potassium tartrate together, what is the pH of the solution given that $pK_{a1} = 3.03$ and $pK_{a2} = 4.37$?

In solutions containing tartaric acid and tartrate ions the following equilibrium equations must apply simultaneously i.e.

$$H_2T \rightleftarrows H^+ + HT^-$$

$$HT^- \rightleftarrows H^+ + T^{--}$$

therefore $$pH = pK_{a1} + \log \frac{[HT^-]}{[H_2T]} \quad \text{..... (I)}$$

and $$pH = pK_{a2} + \log \frac{[T^{--}]}{[HT^-]} \quad \text{..... (II)}$$

where $[H_2T]$ = concentration of undissociated tartaric acid

$[HT^-]$ = concentration of hydrogen tartrate

$[T^{--}]$ = concentration of tartrate

Observe that if you remember the equation as:

$$\text{"pH} = pK_a + \log \frac{[\text{salt}]}{[\text{acid}]}\text{"}$$

Then $[HT^-]$ is the "salt" in Equation I and the **same** $[HT^-]$ is the "acid" in Equation II. The hydrogen tartrate ion, of course, is **simultaneously** both a salt HT^- (of the acid H_2T) and the acid HT^- (which gives the salt T^{--}).

In 0.2 mol/L tartaric acid solutions all three species exist together in equilibrium — undissociated tartaric acid being the most abundant species. In 0.2 mol/L sodium potassium tartrate all three species exist together in equilibrium — tartrate ions being the most abundant species. On mixing the two solutions together a new equilibrium is established and the same equilibrium equations apply **simultaneously** to the new solution. It is irrelevant to even consider where the hydrogen tartrate came from — it simply must exist at a concentration related to the concentration of undissociated tartaric acid, tartrate and pH by the two simultaneous equations (Eq I and II). To solve these simultaneous equations, and determine the pH of the solution; if we have 4 unknowns ($[H_2T]$, $[HT^-]$, $[T^{--}]$ and pH), we need 4 independent simultaneous equations, therefore we require two further equations.

We know that the total amount of tartrate is 0.2 mol/L i.e.

$$[H_2T] + [HT^-] + [T^{--}] = 0.2 \text{ mol/L} \quad \text{..... III}$$

We know that the solution is electrically neutral since lightning bolts do not jump into or out of the solution i.e.

$$[Na^+] + [K^+] + [H^+] = [HT^-] + 2[T^{--}] + [OH^-] \quad \text{..... (IV)}$$

however since

$$[H^+] \times [OH^-] = 10^{-14} \text{ mol/L} \quad \text{..... (V)}$$

The concentration of hydrogen ions and hydroxyl ions is very small compared with the concentration of the three tartrate species (0.2 mol/L) and therefore can be ignored in this example. When dilute buffer solutions (10^{-5} to 10^{-7} mol/L) are considered (or the pH is very high or low) we must use both Equation IV and V in our solution. However in the present example Equation IV simplifies:—

$$[Na^+] + [K] = [HT^-] + 2[T^{--}] \quad \text{..... (VI)}$$

To solve the simultaneous equations:—

Let $[H_2T] = A$, $[HT^-] = B$ and $[T^{--}] = C$

Therefore Eq I becomes:—

$$pH = 3.03 + \log \frac{B}{A} \quad \text{..... (VII)}$$

Therefore Eq II becomes:—

$$pH = 4.37 \log \frac{C}{B} \quad \text{..... (VIII)}$$

Therefore Eq III becomes:—

$$A + B + C = 0.2 \text{ mol/L} \quad \text{..... (IX)}$$

Therefore Eq VI becomes:—

$$B + 2C = 0.2 \text{ mol/L} \quad \text{..... (X)}$$

On rearranging Eq X

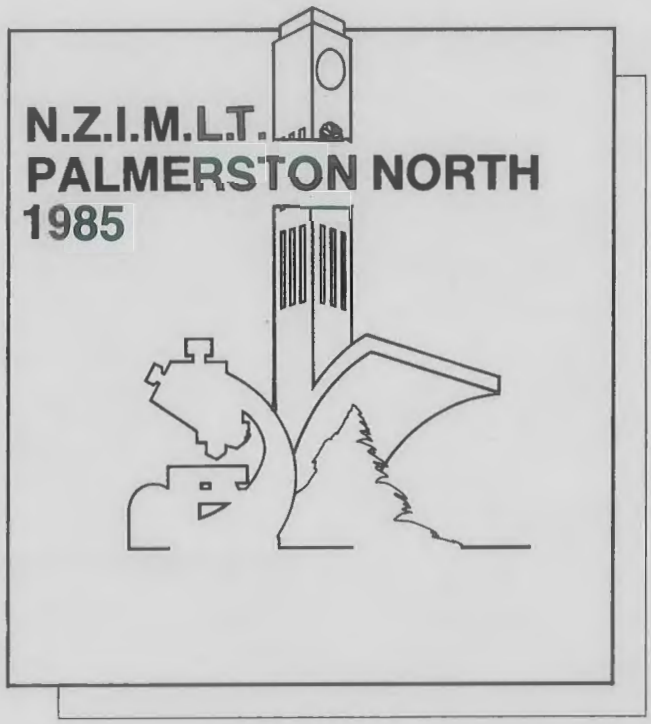
$$C = \frac{0.2 - B}{2} \quad \text{..... (XI)}$$

(to Page 77)

NEW ZEALAND INSTITUTE OF MEDICAL LABORATORY TECHNOLOGY

41st ANNUAL SCIENTIFIC MEETING

Palmerston North



TOWARDS THE YEAR 2000

MASSEY UNIVERSITY

Monday 12th to Wednesday 14th August 1985

Information and Registration Form

Information

Palmerston North
Is New Zealand's second largest inland city and because of its central position is an ideal location for convention activities. Palmerstonians have a cosmopolitan heritage, are friendly, tolerant and proud of their city (it must be right the PRO said it all).

Despite all this it can be cold and wet in the Manawatu in August so we strongly recommend you bring raincoats, umbrellas and good warm spirits with you.

Massey University
Massey Campus is about 5 km from the city centre. The university buildings cover 40 HA so it is possible you may have to walk a few hundred yards while at this meeting. Although Massey was originally an agricultural college neither gumboots or overshoes will be necessary.

The hostels provide student-type single accommodation. Each hostel has a common room, laundry, telephone and ablutions. It is likely that in this enlightened age no segregation of sexes will be permitted in the hostels. Our experience at Dunedin last year would recommend delegates bring dressing gowns. Newspapers will be gratis.

Format of Scientific Meeting
The theme chosen for this years meeting is "Towards the year 2000". In addition to exploring in depth some of the current problems in Medical Technology like AIDS and Extra-Laboratory Testing we have invited some international speakers to engage in crystal ball gazing sessions which may help to suggest the shape of Medical Technology in the year 2000. This will be interesting, informative and will promote animated discussion. This is your profession — if you are interested in your future you owe it to yourself to be at Massey.

The meeting will be structured and most papers will be by invitation. However if anyone is interested in speaking on any of the following topics you are invited to write to the Organising Committee as soon as possible:—

* Extra-Laboratory Testing
* AIDS
* Instrumentation — Towards the Year 2000.
* Data Processing now and in the future.
* Treatment of infectious diseases in the year 2000.

Guest Speakers

Harry L. Pardue
Professor of Chemistry, Purdue, University, USA.
TOPIC — Instrument Developments to the Year 2000.

Al and Helen Free
Senior Scientific Contractors to Miles Laboratories.
TOPIC — Extra Laboratory Testing and Towards the Year 2000.

David T. Durack
MD, D Phil, Professor of Medicine, Microbiology and Immunology. Chief, Division of Infectious Diseases. Duke University, Medical Centre, Durham, D.C.
TOPIC — Towards the Year 2000.

David A. Cooper
B.Sc (Med) M.D F.R.C.P.A., Staff Specialist in Immunology, St Vincents Hospital, Darlinghurst, NSW.
TOPIC — Immunology of AIDS.

Poster Displays — PARTICIPATE — DON'T JUST LISTEN.
Facilities and time will be allocated for poster displays — Subjects may be any facet of Medical Laboratory Technology — not only scientific. If you have a pet scheme for education of Medical Technologists prepare a poster and be prepared to discuss and defend it during refreshment breaks.

POSTERS SHOULD FIT IN 3ft x 2ft SPACE.

Accompanying Persons
A wide range of interests can be satisfied in the Manawatu.

These include the Rose Garden, bush walks, NZ Rugby Museum, shopping, historic homes, craft and antique shops, golf, squash, ten pin bowling.

If there is sufficient interest we will organise a programme — let us know your interest or special requirements on Registration.

Theatre and Restaurants
The city has a range of restaurants catering for all tastes. Centrepoint theatre is a live professional theatre-restaurant. Dinner and show from 6.45 pm fully licenced. Further information will be available in your conference satchel.

MICROCOMPUTER WORKSHOP — Eric Johnston — Auckland
A one day 'hands on' workshop using IBM PC Computer is arranged.

Two courses are offered and will be run concurrently:

1. BASIC PROGRAMMING for beginners. This is based on material used for the last two years at the Auckland Branch workshop. No previous experience with computers is assumed and participants are introduced to the BASIC language, from using the computer as a calculator through to writing and running their own BASIC programs. Simple output to printer is taught as well as elementary disk operation allowing saving and loading programs from diskette.

2. WORD PROCESSING. For those who have some computer familiarity this will provide an introduction to word processing using PEACHTEXT a common a very comprehensive microcomputer package. This course will assume very basic computer/keyboard familiarity although no pogramming skills are required.

Intending participants are requested to indicate any computer skills that they have, particular interest (if any) and the course they would prefer. This information will assist in the selection of material for the workshop.

This course will be run using personal instruction material allowing participants to learn from themselves while actually using the computer. Supervisors will be on hand to assist with any problems. All course material has been tested in use.

3. **PARASITOLOGY WORKSHOP** — Graham Paltridge — Jeff Day
A "hands on" one day workshop with emphasis on the practical side of staining and identifying Intestinal Protozoa. Limited registration.

4. **MICRO ABO Rh BLOOD GROUPING** — Andrew Au
A half day workshop, maximum 20 people. Automated and semi automated Micro ABO and Rh grouping techniques.

HORMONE ASSAY WORKSHOP — Dr John T. France
Amersham Australia Pty Limited in association with the NZIMLT have pleasure in inviting you to their Educational Seminar on Hormone Assays in Reproductive Endocrinology.
The topics to be discussed will include: — Serum Oestriol measurements; Hormone Assays in the Assessment of Infertility; Hormone monitoring of the IVF patient.
Since numbers attending will be limited please register as soon as possible.

On rearranging Eq IX and substituting C with Eq X

$$A = \frac{0.2 - B}{2} \quad \text{..... (XII)}$$

Combining Eq I and II

$$3.03 + \log\frac{B}{A} = 4.37 + \log\frac{C}{B} \quad \text{..... (XIII)}$$

Rearranging Eq XIII

$$\log\frac{B}{B} + \log\frac{B}{C} = 1.34 \quad \text{..... (XIV)}$$

Substituting A by Eq XII and C by Eq XI we get

$$\log\frac{4B^2}{(0.2-B)^2} = 1.34 \quad \text{..... (XV)}$$

this simplifies to

$$2\log\frac{4B}{(0.2-B)} = 1.34$$

which can easily be solved. However in some examples we have to solve a quadratic equation and it is this type of solution which we will now demonstrate

and on taking antilogs of Eq XV we get

$$4B^2 = 21.877(0.2-B)^2$$

which rearranges to

$$0 = 17.877B^2 - 8.7508B + 0.87508$$

on solving this quadratic equation (see Section IV for details) we get

$B = 0.35$ and $B = 0.14$ mol/L

However since B must be less than 0.2 there is only one practical solution i.e. $B = 0.14$ mol/L

therefore $A = 0.03$ mol/L and $C = 0.03$ mol/L

By substituting in either Eq I or II pH = 3.70

Actually in this example since we are using equal volumes of equimolar solutions of tartaric acid and sodium potassium tartrate the calculation can be simplified if it is realised that:

$$[H_2T] = [T^{--}]$$

(Can you see why this is so? — look at the dissociation equations again.)

Eq I rearranges to:—

$$pH = pK_{a1} + \log[HT^-] - \log[H_2T]$$

Eq II rearranges to:—

$$pH = pK_{a2} + \log[T^{--}] - \log[HT^-]$$

on adding together

$$2pH = pK_{a1} + pK_{a2} + \log[T^{--}] - \log[H_2T]$$

since $[H_2T] = [T^{---}]$

then $2pH = pK_{a1} + pK_{a2}$

therefore $pH = \frac{pK_{a1} + pK_{a2}}{2}$

therefore $pH = \frac{3.03 + 4.37}{2}$

$= 3.70$ (the same answer)

Example:— *Calculate the pH of a Buffer solution used in Serum Protein electrophoresis*

Chemical Composition

Sodium 5,5-diethylbarbituric acid (molecular weight 206)	47.5 g
5,5-diethylbarbituric acid (molecular weight 184)	15.0 g
($pK_a = 7.43$)	
Tris (hydroxymethyl) amino methane (molecular weight 121)	23.0 g
($pK_a = 8.08$)	
Distilled water q.s.	1000 mL

Hints on solving the problem

Acid dissociation of Barbiturate:—

$$BH \rightleftarrows H^+ + B^-$$

therefore $pH = 7.43 + \log\frac{[B^-]}{[BH]}$ ..... (I)

Acid dissociation of the salt of Tris:—

$$TH^+ \rightleftarrows H^+ + T$$

therefore $pH = 8.08 + \log\frac{[T]}{[TH^+]}$ ..... (II)

The total barbituric acid/barbiturate concentration is:—

$$BH + B^- = \frac{47.5}{206} + \frac{15.0}{184} \quad \text{..... (III)}$$

$$= 0.313 \text{ mol/L}$$

The total Tris concentration is:—

$$TH^+ + T = \frac{23}{121} \quad \text{..... (IV)}$$

$$= 0.190 \text{ mol/L}$$

The solution must be electrically neutral therefore

$$Na^+ + TH = B^-$$

since $Na^+ = \frac{47.5 \text{ mol/L}}{206}$

$$0.23 + TH^+ = B^- \quad \text{..... (V)}$$

There are 5 unknowns i.e. pH, [BH], $[B^-]$, $[TH^+]$ and [T] and we have 5 independent simultaneous equations, therefore we can determine the pH of the buffer.

Metal Ion Buffers

Buffering of ions in solution is not restricted only to hydrogen (or hydroxyl) ions.

For example in the determination of alkaline phosphatase some workers have buffered the concentration of Mg^{++} and Zn^{++} ions by using HEDTA to chelate Mg^{++} and Zn^{++} ions thus maintaining a constant and optimum concentration of Mg^{++} and Zn^{++} for enzyme activation[1].

In the measurement of plasma magnesium, calcium interference has been minimised by using strontium EGTA to maintain a low level of free EGTA ions to bind calcium which also responds as magnesium in the assay[2].

References

1. Teitz N.W. A Reference Method for Measurement of Alkaline Phosphatase Activity in Human Serum. *Clin Chem* 1983; **29**: 751-761.
2. Gitelman J.H., Hunt C. and Lutwak L. An Automated Spectrophotometric Method for Magnesium Analysis. *Anal Biochem* 1966: **14**: 106-120.

Problems (Answers page 90)

* Model answers available from authors on request

1. Assuming the effective buffer range of a weak acid or salt of weak base is given by $pH = pK_a \pm 1$, what effective buffers can be prepared from the following?
   a) Formic Acid $pK_a = 3.8$
   b) TRIS $pK_b = 5.9$
   c) AMP $K_b = 5.01 \times 10^{-5}$
   d) Glycine $pK_{a1} = 2.4$, $pK_{a2} = 9.8$
   e) Phosphate $pK_{a1} = 2.1$, $pK_{a2} = 7.2$, $pK_{a3} = 12.3$
   f) Citric Acid $K_{a1} = 7.9 \times 10^{-4}$, $K_{a2} = 1.58 \times 10^{-5}$, $K_{a3} = 3.98 \times 10^{-7}$
   g) Tartaric Acid $pK_{a1} = 3.0$, $pK_{a2} = 4.4$
2. a) 100 mL of 0.1 mol/L hydrochloric acid is diluted to 1 litre with distilled water. Calculate the pH of the hydrochloric acid solution before and after dilution.
   b) 100 mL of 0.1 mol/L acetate buffer pH 4.8 is diluted to 1 litre with distilled water. Given that the pK_a of acetic acid is 4.8 calculate the pH of the acetate buffer before and after dilution. (Note — refer to the Henderson-Hasselbalch equation.)

3. 10 mL of 1 mol/L hydrochloric acid is added to 1 litre of 1 mol/L acetate buffer pH 4.8 and to 1 litre of 0.1 mol/L acetate buffer pH 4.8. Calculate the change in pH of both buffers and show that the more concentrated buffer has the greater buffering capacity.

4. How would you prepare 300 mL of an acetate buffer pH 4.8 from the following solutions? In each case give the total acetic acid/acetate concentration of the buffer.
a) 1 mol/L hydrochloric acid and 1 mol/L sodium acetate.
b) 1 mol/L acetic acid and 1 mol/L sodium acetate.
c) 1 mol/L acetic acid and 1 mol/L sodium hydroxide.

5. Calculate the pH of 100 mL of 0.1 mol/L acetic acid solution after the addition of
a) 25 mL of 0.1 mol/L sodium hydroxide
b) 50 mL of 0.1 mol/L sodium hydroxide
c) 75 mL of 0.1 mol/L sodium hydroxide

6.* A solution prepared by mixing 100 mL of hydrochloric acid (0.2 mol/L) and 100 mL of acetic acid (0.2 mol/L) is mixed with 200 mL of sodium hydroxide solution (0.3 mol/L). Calculate for the mixture:—
a) the concentration of Na^+.
b) the concentration of Cl^-.
c) the concentration of acetate ions.
d) the concentration of hyroxyl ions.
e) the concentration of hydrogen ions.
Given that the acid dissociation constant for acetic acid is 4.8.

7.* Tris-(hydroxymethyl amino methane) (12.1 g) is weighed into a litre beaker and approximately 500 mL of distilled water is added. After all the tris has dissolved, 1 mol/L hydrochloric acid solution is added dropwise till the pH of the mixture is 7.50. The mixture is now transferred to a 1 litre volumetric flask and made up to the mark. Molecular weight tris- (hydroxymethyl amino methane) = 121.
$pK_a = 8.08$.
Calculate
a) the molar concentration of tris in the resulting buffer solution.
b) the molar concentration of tris hydrochloride in the buffer solution.
c) the volume of hydrochloric acid solution which has been added.

8.* To 30 mL of 0.2 mol/L tris- (hydroxymethyl amino methane) solution is added 20 mL of 0.2 mol/L hydrochloric acid solution. In the resulting solution, given the pK_a of tris-(hydroxymethyl amino methane) hydrochloride is 8.08, what is/are:
a) the molar concentration of chloride ions?
b) the molar concentration of unionised tris-(hydroxymethyl aminomethane)?
c) the molar concentration of protonated tris-(hydroxymethyl aminomethane)?
d) the molar concentration of hydrogen ions?

9.* To 50 mL of 0.1 mol/L butyric acid solution is added 10 mL of 0.3 mol/L sodium hydroxide solution. Given that the pK_a for butyric acid is 4.82 what is/are:
a) the molar concentration of sodium ions?
b) the molar concentration of butyrate ions?
c) the pH?

10. Calculate the hydrogen ion concentration in a phosphate buffer pH 7.3 prepared by mixing 0.2 mol/L NaH_2PO_4 with 0.25 mol/L K_2HPO_4. (The acid dissociation constants for phosphoric acid are $pK_1 = 2.1$, $pK_2 = 7.2$ and $pK_3 = 11.8$). Did the solution of this problem take longer than 60 seconds?

11.* In plasma, uric acid is referred to as plasma uric acid and sometimes as plasma urate. Given that the acid dissociation constant for uric acid is 3.9, which of these species would generally be the more accurate description at physiological pH (e.g. 7.4)?

12.* In text books, an important intermediate of the tricarboxylic acid cycle is sometimes called succinic acid or succinate. At physiological pH (e.g. 7.4), which of these species would generally be the more accurate description? Given that the acid dissociation constants for succinic acid are $pK_1 = 4.21$ and $pK_2 = 5.64$.

13.* Calculate the pH of 100 mL of 0.5 mol/L glycine solution after the addition of
a) 30 mL of 1 mol/L hydrochloric acid and
b) 30 mL of 1 mol/L sodium hydroxide solution.
Given the acid dissociation constants for glycine are $pK_1 = 2.4$ and $pK_2 = 9.8$.

14.* Tartaric acid (15.0 g) is weighed into a litre beaker and approximately 500 mL of distilled water is added. After all the tartaric acid has dissolved, 1 mol/L sodium hydroxide solution is added dropwise till the pH of the mixture is 3.80. The mixture is now transferred to a 1 litre volumetric flask and made up to the mark. Tartaric acid molecular weight 150, $pK_{a1} = 3.03$, $pK_{a2} = 4.37$.
Calculate
a) the molar concentration of tartaric acid in the resulting buffer solution.
b) the molar concentration of the singly and doubly ionised tartrate ions.
c) the volume of sodium hydroxide solution which has been added.

15.* Equal volumes of 0.1 mol/L trisodium citrate and 0.1 mol/L citric acid are mixed. What is the pH of the final solution, given that $pK_{a1} = 3.1$, $pK_{a2} = 4.8$, $pK_{a3} = 6.4$?

16.* Calculate the pH of a buffer solution used in Serum Protein electrophoresis — see last example for hints on solving this problem.
Chemical Composition

Sodium 5,5-diethylbarbituric acid (molecular weight 206)	47.5 g
5,5-diethylbarbituric acid (molecular weight 184) ($pK_a = 7.43$)	15.0 g
Tris (hydroxymethyl) amino methane (molecular weight 121) ($pK_a = 8.08$)	23.0 g
Distilled water q.s.	1000 mL

WELCOME TO STOCKHOLM

The 17th Congress of the International Association of Medical Laboratory Technologists Stockholm 3—8 August, 1986

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- AN EXHIBITION OF EDUCATIONAL MATERIAL USED IN THE TRAINING OF MEDICAL TECHNOLOGISTS AND RELATED HEALTH PERSONNEL
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The Pacific Way

The Pacific Paramedical Training Centre A Five Year Perspective

Ron MacKenzie, Ph.D., D.H.A., F.N.Z.I.M.L.T.

The Pacific Paramedical Training Centre (P.P.T.C.) is now beginning its fifth year of operation and this seems an appropriate time to look forward and make plans for the future. But in doing this it is important to look back and review development during the foundation period.

First, a brief description of the Pacific Paramedical Training Centre, what it is and what it is attempting to do. The Centre is located at Wellington Hospital and is dedicated to the promotion of appropriate health technology throughout the Pacific Region. The Centre is a non-profit organization supported by the New Zealand Ministry of Foreign Affairs, the Department of Health, the New Zealand Red Cross Society, the New Zealand Institute of Medical Laboratory Technology and the World Health Organization. Generous support has also been received from a number of Rotary Clubs throughout New Zealand.

The Centre has a steering committee of five, representing the Department of Health, External Aid Division of the Ministry of Foreign Affairs, the New Zealand Institute of Medical Laboratory Technology, Wellington Hospital and the Red Cross Society. Dr H.C. Ford representing Wellington Hospital and Dr R. Mackenzie representing the New Zealand Red Cross Society act as co-chairmen and the committee secretary is Mrs B. Gunn of the International Division, Department of Health.

While the role of the centre is the promotion of appropriate training in any of the paramedical disciplines that may be requested by the Pacific Island countries, the initial thrust of the Centre has been, and continues to be in the field of medical laboratory technology. Since its inception in 1981 some sixty-five trainees from laboratory services of the Pacific have attended courses of three months duration at the Centre, mainly in the areas of Water and Food Microbiology, Blood Bank Technology/ Haematology and the laboratory diagnosis of acute diarrhoeal and respiratory infections. Trainees attending these courses have been funded in the following ways: W.H.O. Fellowships, the New Zealand Government Bilateral Aid Programme, and the New Zealand Red Cross Society's Health Science Award Scheme. One trainee has been funded by the New Zealand Federation of University Women, and beginning in 1985 the League of Red Cross and Red Crescent Societies, based in Geneva, have agreed to fund four trainees annually to a blood bank technology course. One important development which took place in 1984 was the inception of an ongoing microbiology quality control programme for the Pacific Islands. This was requested by W.H.O. as a follow-up mechanism for trainees who have attended microbiology courses at the Centre. The programme is carried out jointly by the Centre and the National Health Institute and is seen as an excellent way of maintaining contact with former trainees and maintaining standards. It is intended to extend this programme into other disciplines of medical laboratory technology in the future.

In reviewing the work of the centre during its first five years, two things must be acknowledged. Firstly, that much of what has been achieved during this period has been due to the enthusiasm and hard work of Mrs Andrea Hall, the first tutor/co-ordinator who left the Centre in December, 1984, to take up residence in Auckland. The Committee have been most fortunate in obtaining the services of Mr M.J. Lynch to succeed Mrs Hall. Mr Lynch is known to many Institute members as a Technologist with wide experience in both the practical and teaching aspects of medical laboratory technology. (See article Vol. 38. 1984, p.137.)

Secondly the centre is most appreciative of the equipment which has been donated for use in the teaching laboratory by both hospital and private laboratories throughout New Zealand, and to Wellington based technologists who have contributed freely with their teaching skills. Special thanks are extended to Marilyn Eales, N.Z.I.M.L.T. representative on the P.P.T.C. Steering Committee for her much valued advice over the past five years.

What then of the next five years; what of a philosophy for the future? Is a change of direction required or should we be like a coral reef with peripheral growth only and no reshaping of the old and established? Clearly the Centre must continue to develop, but like a Pacific coral reef the development and growth should be on the outer edges. I believe we have established in the first five years a sound foundation to which we can now add as required.

In approaching the next five years in the Centre's life an important question must be asked . . . Are we getting a good return on the training dollars invested? If this question is approached on a cost-benefit basis in which the monetary cost of the teaching programmes is compared with the expected benefits, normally these benefits are expressed in dollars; and I use the word "normally" to indicate that there are exceptions because costs, benefits or both can be expressed in non-dollar terms. In dollar terms the first five years of the Centre's life must be seen as cost beneficial if "benefits" are taken as the number of people trained in specific laboratory skills and "costs" are related to the number of trainers needed to achieve this. In non- dollar terms the expected benefits may be seen as improved work performance when improved, new and appropriate laboratory techniques are introduced on the trainees return home. This criterion judged by reports from laboratory supervisors and follow-up visits from W.H.O. officers and others, does indicate that the P.P.T.C. teaching programmes have been largely cost beneficial. The application of quality control programmes will however be the final arbiter of this and it should be remembered in all technical training and development programmes that — "you win a few, you lose a few".

If the value of the Centre's work was considered on a cost-effectiveness basis a different approach must be used. Costs must be calculated and alternate ways compared to achieve the same set of results. In essence if the centre was seeking the best way to train overseas laboratory workers in specific skills by either bringing them to Wellington or by sending trainers to them, accurate cost comparisons must be obtained — this would be described as a study in cost effectiveness. This last point, that of sending experts to areas where laboratory upgrading is required or bringing laboratory personnel to New Zealand is one which has evoked considerable discussion by the N.Z.I.M.L.T. Clearly, both methods can be cost beneficial and cost effective depending on how and where they are used. They are complimentary training approaches. In regard to this, the N.Z.I.M.L.T. must be commended on the present initiatives it is taking in attempting to set up short term technical training assignments for New Zealand Medical Laboratory Technologists as part of its overseas aid programme. If this scheme can be achieved it would be complimentary to the work of the P.P.T.C. and make a significant contribution to strengthening the medical laboratory services of the Pacific Region.

Finally, the goal of the P.P.T.C. remains the same as it enters the second five years of its existence — the provision of short term technical training programmes designed to meet the needs of each overseas trainee and his/her laboratory, with an emphasis on appropriate and practical training that they can use immediately upon returning home.

Summary of Work Performed by Laboratories — 1982

	Central	Lautoka	Labasa	1982 Total	1981 Total	1980 Total
1. Haematology	132,112	93,475	18,126	243,713	216,366	175,025
2. Blood Transfusion	50,152	92,804	7,145	150,101	134,572	111,608
3. Biochemistry	99,973	57,219	11,369	168,561	159,065	112,743
4. Micro-biology, Serology and Parasitology	126,732	42,375	8,275	177,382	161,859	134,555
5. Histology, Cytology, Autopsies and Forensic Medicine	21,044	16,503	1,402	38,949	35,708	17,263
TOTAL	430,013	302,376	46,317	778,706	707,570	551,194

Medical Technology in Fiji

At the invitation of the Fiji Medical Laboratory Technologist Association I attended the first seminar of the association in Suva on Saturday December 15th. The first annual general meeting of the association followed on the 16th December. The theme for the seminar was Recent Trends in Laboratory Medicine and as keynote speaker I discussed some of the recent trends in New Zealand including instrumentation, accreditation and quality assurance, extra laboratory testing and AIDS. I had been concerned that some of the topics were possibly inappropriate however these fears disappeared when I saw the programme and heard the other speakers.

The local organisations were very pleased with the support (about 70) especially from people who had travelled from Lautoka and Labasa. I could find no fault with the arrangements which followed closely any similar seminar in New Zealand. The social arrangements also were very similar, with surprisingly large quantities of Fiji bitter being loaded for the harbour cruise and none being returned.

Laboratory Division

"The two main Clinical Laboratories are located at the Colonial War Memorial Hospital and Lautoka Hospital. These two laboratories, apart from serving the needs of the two large hospitals, are also referral laboratories for the rest of Fiji. They are administered by two Consultant Pathologists. Labasa Hospital has limited laboratory services provided by a team of laboratory technicians under the control of a Senior Technical Officer. Basic laboratory services are also available at sub- divisional hospitals and health centres which are mostly provided by nurse technicians. These officers are trained to conduct simple laboratory procedures as well as taking simple x-rays.

The main responsibility of the laboratory division is to provide support services to all the hospitals in Fiji. In addition to this responsibility they also operate as public health laboratories, where testing of food and water samples are carried out according to the Pure Food Regulations requirements. Chemical analysis of food samples are conducted by the Government Analyst of the Ministry of Agriculture and Fisheries. Both the Colonial War Memorial Hospital and Lautoka Hospital Laboratories continue to provide forensic services to the Royal Fiji Police Force. This service is becoming a highly specialized branch of medicine and time has now come to seriously consider its separation from the Clinical Laboratory service. More complicated laboratory examination procedures which are not available in Fiji are conducted overseas where specimens are sent by special air-freight.

As laboratory services are essential diagnostic tools, the Ministry of Health is endeavouring to develop the Labasa Hospital Laboratory to the same standard as the Colonial War Memorial and Lautoka Hospitals. However, efforts will be directed towards training manpower and procurement of adequate equipment for this Laboratory. As mentioned in the 1981 Annual Report the need to train more Laboratory Technicians has become an urgent issue in order to develop the laboratory services at sub-divisional hospitals and major health centres in Fiji. The detail of activities of the Laboratory Division during 1982 is shown.

Virus Laboratory

This laboratory is located in a self-contained unit in Tamavua Hospital under the control of the Government Virologist. The laboratory is mostly involved with research into the wide field of Virology. It continued to monitor Influenza, Dengue Fever, Ross River Fever and other viral infections prevalent in Fiji. It also acted as a World Health Organisation Dengue Fever Referral Centre for the South-West Pacific Region during the year. The laboratory worked very closely with other Virology laboratories in other countries.

The total number of tests conducted by the Virus Laboratory for Diagnostic Serology for 1982 was 13,561 at the same time there were 4,978 tests carried out at the centre for weekly Influenza surveillance. The Virologist is also responsible for the National Filariasis Campaign. During the year under review, he conducted several field surveys to determine the filariasis density in the community, before Mass Drug Administration is carried out."

The main causes of death now are diseases of the circulatory and respiratory sysems, cancer and accidents i.e. similar to other western developed civilisations and demonstrating a reversal from infectious disease to non communicable disease. Small outbreaks of dengue fever, measles and chicken pox occur. Leprosy and tuberculosis are under control, however sexually transmitted diseases have become a major social disease. Malnutrition, both undernourishment due to protein deficiency in children, and overnourishment in the adult leading to obesity and overweight, are also problems.

Total Health expenditure in 1982 was 26 million Fiji dollars ($F 1 = $NZ 2) for a population of 663,000; compare this with the total payments to the NZ Public Hospital Service of 652 million dollars in the year ended March 1984, i.e. the equivalent of 326 million Fiji dollars for a population about 5 ½ times greater and this New Zealand figure does not take account of private laboratory payments.

I was impressed by the quality and understanding of the Medical Technologists I met. Many have had overseas training and experience. Generally speaking methods and equipment seemed adequate. Most of the real problems come from inadequate funds being available to provide adequate Quality Control material in all laboratories. Diagnostic kits are not always available; for example all blood donors were not routinely tested for Hepatitis B as the kits were not available. There is also a shortage of recent text books and journals.

The establishment of a professional association will, I am sure, eventually solve some of the problems staff have with working conditions and salaries. I will be maintaining contact with the Technologists of Fiji especially their President Satish Sudhailar and Secretary Rajendra Parmar. The association is to hold a mini seminar on 13th July 1985 on the Laboratory Investigation of Sexually Transmitted Disease and the Annual Scientific Meeting will be in November.

I would like to thank the Fiji Medical Technologists Association for the invitation to be present at their first Annual Conference.

Reference

Ministry of Health Annual Report for the Year 1982. Parliament of Fiji. Parliament Paper No. 27 of 1984.

Colvin Campbell

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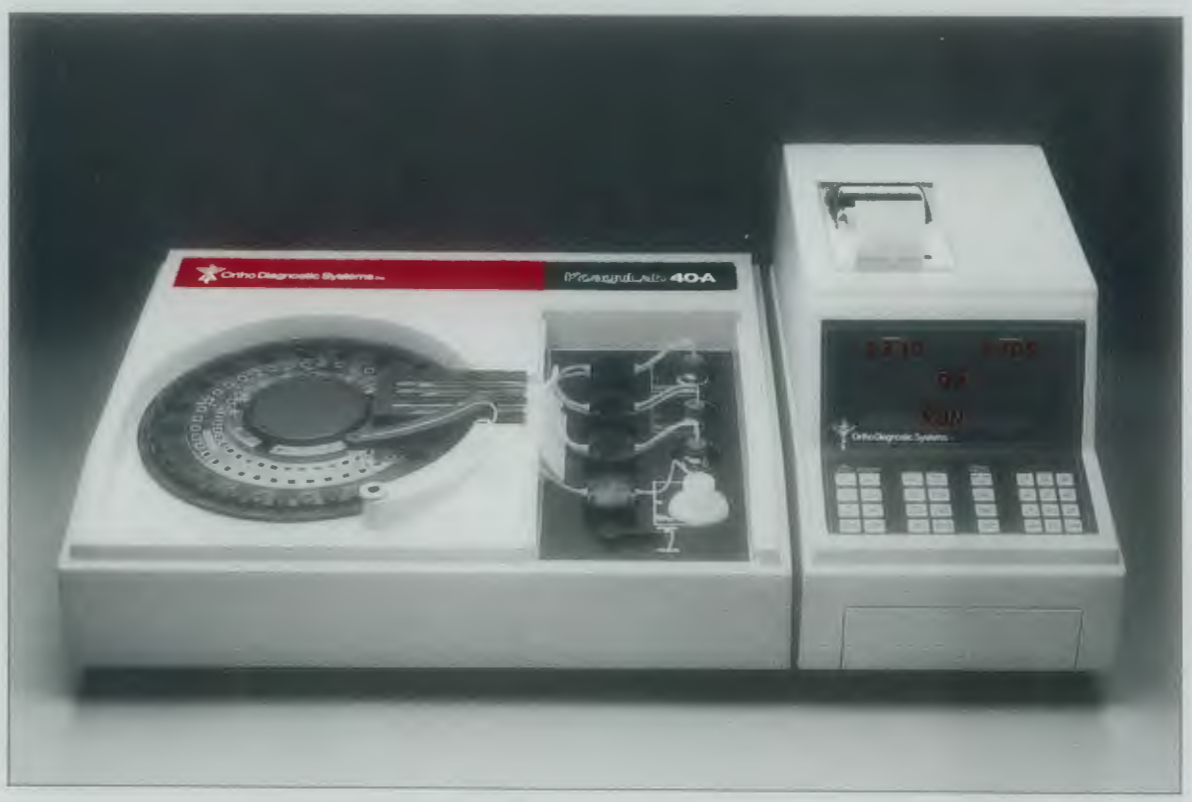

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Circle 41 on Reader's Reply Card

LABORATORY SAFETY — AIDS

Recent publications on AIDS have lead to considerable discussion and concern by many people in a variety of professions. As a result of this highly emotive issue and the many misconceptions about AIDS the Council of the N.Z.I.M.L.T. has established a Task Force on AIDS with specific terms of reference.

Until the Task Force has addressed the problem fully the following general comments are for the attention of **ALL LABORATORY WORKERS.**

The causitive agent of AIDS, HTLV3 is not highly infectious. It is spread mainly by homosexual intercourse, and by blood and blood products in a similar way to hepatitis B. There is no evidence that it is spread by casual contact, by food, sweat, saliva or by airborne routes.

In New Zealand there is no data on the incidence of carriers or those infected, and there will be some considerable time lapse before these figures become available.

The following basic precautions are relevant in light of current knowledge.

1) Treat **all** specimens as potentially infectious.
2) Maintain high standards of personal hygiene (hand washing).
3) Wear protective clothing when handling large numbers of blood specimens.
4) Protect open wounds.
5) Prevent wounds from sharp instruments and needles.
6) Dispose of contaminated articles appropriately.
7) Clean up all blood spills promptly, using an approved disinfectant.
   e.g. (i) 0.5-1.0% sodium hypochlorite.
   (ii) 40% aqueous formalin.
   (iii) 2% glutaraldehyde.
8) Use disposable gloves for any procedure which may appear hazardous.
9) Disinfect work surfaces regularly.
10) Be ALERT and stay INFORMED.

IN THE CASE OF A CONFIRMED OR SUSPECTED AIDS CASE FOLLOW HEPATITIS-B PROCEDURES.

1. Avoid direct contact of your skin and mucous membranes with blood, blood products.
2. Avoid splashing blood in the eyes or mouth.
3. Avoid shaking or homogenizing.
4. Take care when opening sealed containers or bottles.
5. Protective clothing including gloves and facial covering (if danger of splashing) should be used.

Careful technique and good personal practice should be followed at all times.

There are no known cases of health-care personnel or other care providers contacting the illness by caring for AIDS patients. However, until further information on the New Zealand scene is available the utmost care is required in handling all specimens.

B. CORNERE
Convenor,
Special Task Force On Aids.

ELI LILLY MICROBIOLOGY SCHOLARSHIP

This award, consisting of $500 kindly donated by Lilly Industries (NZ) Ltd, is to be used **either** for the purpose of funding a research project which cannot otherwise be undertaken or to attend an overseas scientific meeting. The scholarship is open to all financial members of the NZIMLT currently working in the field of Microbiology. Applicants for the Scholarship must apply on the official application form available from the Secretary of the NZIMLT.

Acceptance of the Scholarship will require the recipient **either** to prepare an article for publication in the NZIMLT journal relating to that research **or** prepare a full report on the meeting attended for publication in the NZIMLT journal.

Applications close on **1 July 1985** with the Secretary, NZIMLT, Haematology Dept, Christchurch Hospital, Christchurch. The successful applicant will be announced at the Annual Scientific Meeting.

Letters To The Editor

Re: New Science T.V. Programme
Dear Sir,

This year Television New Zealand is launching a new science and technology programme to be screened on Saturday nights at 7 p.m.

The programme is called "Fast Forward", and will be produced out of our Christchurch studios.

It will combine the best aspects of "Science Express" and "Production Line". "Fast Forward" will have a popular approach to the latest technology being developed both in New Zealand and overseas. The programme will also cover all areas of scientific investigation and discovery.

We are particularly interested in any new discoveries or breakthroughs that have an impact on the community.

Could you please send us an update on what your organisation is doing. Also, we would appreciate being put on mailing lists for any information or publicity you send out next year.
Thanking you
Regards

"Fast Forward" staff

Any information that members think might be of interest should be sent to the Secretary, Haematology Dept, Christchurch Hospital, Christchurch.

Extra Laboratory Testing
Dear Sir,

Your editorial on E.L.T. in the November, 1984 Journal issue was both thought provoking and timely. Your comments and those given by our immediate Past-President, Mr A.F. Harper in his Presidential Address together focussed on what is surely the major threat our profession will face over the next few years.

I would however like to take issue with your belief that a total ban on all E.L.T. procedures (except the traditional ward urine tests) is what we should be aiming for. Firstly, I believe this is an unobtainable goal. To achieve a total ban, we should have started over ten years ago. Instead, we have become obsessed with work volumes and automation and as a result we have not been providing the other service that was obviously required namely, quick one off test procedure results. As the demand for this grew and the laboratories appeared reluctant to cater for the quick one off tests, the development companies saw a market and quite correctly produced products to achieve these demands. These procedures are here now and are very sophisticated. They will not go away and no amount of regulation is going to make much difference as the problem is still there, namely laboratories find it difficult to compete with the quick one off ward test procedure.

Also, I am fascinated why you single out the "traditional ward urine test" as the one E.L.T. procedure that can remain. If you are so convinced that there should be a total ban on E.L.T. why should urine testing not be included? It can not be because of simplicity as many E.L.T. procedures are equally simple. It should not be because of the large volume because if these were all done in the laboratory then we could create more jobs. And surely your reason can not be because the results of urine dip sticks are not all that important because this could mean that many other laboratory tests are equally useless. (Maybe they are!)

I am convinced that our only option is to take control of the E.L.T. procedures by accepting that what we have "given away" in the past are now irretrievable. Equally, we must assume responsibility for all laboratory test results performed by anyone, anywhere for either screening or diagnostic purposes. To do this we will need to train the operators, control the procedures and maintain all equipment. Be they doctors, nurses or patients, we should assume responsibility for the results that they achieve. We need to get out of the laboratory and see just what is going on out there and offer our expertise to remedy the situation. Who knows, we may even start to find out what services we should be offering rather than telling the consumers what they can have.
Yours sincerely,

Paul McLeod
Nelson Hospital

Institute Membership
Dear Sir,

In a letter published in the Institute Journal November 1984, I criticised the apparent unwillingness of the NZIMLT to recognise the laboratory assistants as a group deserving of, at least, equal consideration with respect to negotiations, conditions of employment, career structures and so forth. I warned that we ran the risk of losing those people from our organisaion for good if it did not.

Members will no doubt be aware that a fledgling organisation, the Medical Laboratory Assistants Union, has been formed with in excess of one hundred laboratory assistants already signed up in the Auckland area.

The purpose of my original letter was to draw to the attention of Council the dissatisfaction of laboratory assistants, not just in the Auckland area but throughout the country as a whole, and, hopefully to generate feedback through these columns, be it critical or supportive. The report of the November Council meeting prepared by our regional representative, states that Council plans to circulate throughout all laboratories a questionnaire aimed at establishing the needs and aspirations of laboratory assistants. Whilst Council's "initiative" is to be applauded, I suggest the exercise is about three years late, judging by developments outlined above.

The minutes of the Special General Meeting held in Dunedin last year, have been published in the November 1984 Journal. I invite ALL laboratory assistants, members and non-members alike, to read those minutes particularly the remits, referred to in my previous letter, applying to laboratory assistants (remits 8 and 9).

Interestingly, after the counting of hands and proxies only one hundred and seventy three (173) and one hundred and eighty (180) votes were cast for each of these remits. Out of a total membership of 1342, only 180 members bothered to vote. Assuming, as I have, that the majority of those attending the meeting were technologists what happened to the other 1162

voting members; particularly what happened to the votes of the 334 laboratory assistant members.

Those laboratory assistants who criticise the Institute for its lack of interest in laboratory assistant affairs, but then throw away the opportunity to change "THE SYSTEM" through the ballot box, deserve little sympathy. Had those 334 members voted, remits 8 and 9 would have been passed and passed handsomely.

Rather than forming an alternative organization, why not change the existing organization. Look at the "Election of Officers". All Council members elected UNOPPOSED. Where was the laboratory assistant nominated to stand as an ordinary member in the Auckland region, Central North Island, Wellington, Christchurch or Dunedin?

The above-quoted voting figures are not unique to this years General Meeting. Examination of previous years S.G.M's voting figures point to an equally pathetic response. "Bunch of jellies" — Janet Marshland, you're so right!

The strength of a profession lies in its professional body. The strength of that body lies in the support and involvement of its members. Laboratory assistants and technologists, JOIN, PARTICIPATE, BE HEARD.

Howard C. Potter
FNZIMLT

Regulation Abuse

Dear Sir,

I am writing to record my concern of the abuse of the regulations covering the conditions of employment of laboratory workers as defined in HS 19 and HS 48. At all meetings of laboratory workers we hear of continual complaints that the Hospital Board's vary in their interpretation of the conditions of employment but very little is heard about the way in which laboratory workers themselves go along with these practices when it suits them.

The particular points of concern are—

1. The observance of the hours of work.
2. The practice of taking time in lieu of overtime worked.

The observance of the conditions defined for hours of work is probably the most widely abused and as defined in HS 19 it means that all laboratory workers who are fulltime employees must work five consecutive days of 8 hours per day. It is still relatively widespread practice for laboratories to place staff on rosters which provide for broken weeks i.e. spells of less than five consecutive days or even worse allowing staff to accumulate "days off" while paying penal time when Saturdays and Sundays are worked whether or not in that week (Monday to Sunday) the individual has worked more than 40 hours. In this situation the employee must be paid overtime for any time worked in excess of five consecutive 8 hour days (refer HS 48 section 3. 1 (B)). There is no other legal interpretation.

I quote from a memorandum from one of the larger Hospital Board's who had this matter drawn to their attention by the Auditors.

"The provisions outlining hours of work, overtime and penal time cannot be departed from regardless of any requests or concurrence of staff."

None of these determinations (HS 16, 19 & 38) allow for taking time off in lieu of overtime worked or allow for the working of less than "the ordinary weekly hours of work". There is nothing that permits a Board to relax or introduce a flexible work pattern to circumvent the provisions laid down. Anything that is in conflict or contrary to the conditions outlined in the determination is beyond the Board's authority and cannot be condoned."

Thus it is clear that all persons have the right to be paid for the actual hours that they are required to work in strict accordance with the regulations and if they falsify their time sheets to conform with any practice be it suggested in any form can lead to the dismissal of the employee as a State Servant.

We hear comments "working to rule" etc, I suggest that before anyone can even contemplate such actions whatever that may mean they must first ensure that they are working to the regulations and that they have the legal right to expect the employer to pay them in strict accordance with the same regulations.

Yours Sincerely,

W.J. Wilson

INSTITUTE BUSINESS

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or the Editor, P.O. Box 35-276, Auckland, 10.

Membership Secretary
David Pees
P.O. Box 29-115, Greenwoods Cnr, Auckland.

Membership Fees and Enquiries

Membership fees for the year beginning April 1, 1985 are:
For Fellows — $45

For Associates — $45

For Members — $30

For Non-practising Members — $20

All membership fees, changes of address or particulars, applications for membership or changes in status should be sent to the Membership Secretary at the address given above.

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

Membership Sub-Committee Report March 1985

Membership

Since our November meeting there have been the following changes:

		Nov 84
Membership as at 13/03/85	1411	1406
LESS resignations (7), G.N.A. (2)		
Deletions of Unfinancial Members (130)	139	18
	1272	1388
PLUS Membership Applications (101)	101	23
TOTAL MEMBERSHIP:	1373	1411

Applications for Membership as at 9 March 1985

Beanland, M.D., Auckland; Belcher, C.D., Auckland; Birch, S.W., Auckland; Bolton, P.F., Hawera; Butler, C.M., Palmerston North; Chapman, M.L., New Plymouth; Chase, K.M., Auckland; Clearwater, S.M., Invercargill; Collman, A.E., Auckland; Cunnington, J.E., Ashburton; Donoggio, J.M., Christchurch; English, P.J., Auckland; Excell, A.M., Auckland; Ferguson, J.K., Hamilton; Fisher, E.T., Dunedin; Ganley, M.E., Auckland; Gay, N.K., Thames; Griffiths, R.J., Auckland; Grimwood, G.A., Auckland; Groom, L.C., Chritchurch; Haigh, J.M., Auckland; Harrison, V.C., Auckland; Hearne, C.J., Auckland; Hensch, T.I., Wellington; Holloway, L.M., Auckland; Hurren, P.J., Auckland; Inglis, K.A.L., Christchurch; James, Y.I., Nelson. Johns, R.J., Auckland; Karl, L.M., Auckland; Kilty, R.A., Christchurch; Kyle, S.M., Dunedin; Leydon, K.M., Auckland; Mackay, D.A., Christchurch; Mackay, E.D., Wellington; Manuel, R.D., Auckland; Marfell, W., Christchurch; Martin, F.G., Hamilton; Mason, L.J., Auckland; McHaffie, D.A., Christchurch; McMaster, S.B., Hamilton; Meisner, A.B., Auckland; Montford, L.E., Auckland; Myles, J.M., Auckland; Norris, J.M., Hamilton; Oakley, A., Hamilton; O'Brien, L.J., Auckland; Pamplin, W.F., Auckland; Perry, J.J., Auckland; Purdy, G.D., Auckland; Ratcliffe, J., Lower Hutt; Reed, M.R., New Plymouth; Reeve, D.M., Invercargill; Ross, S.L., Hamilton; Rowan, C., Rotorua; Rowe, C.G., Auckland; Roycroft, S.J., Christchurch, Rush, A.M., Gisborne; Couzins, J.A., Whangarei; Hunter, J.A., Auckland; Kramer, J.A., Whakatane; Junge, J.C., Auckland; Thornton, A.M., Christchurch; Burrows, L.M., Christchurch; Horne, J.E., Whangarei; Lim, Beng Yim, Malaysia; House, L.A., Perth; Christmas, R., Gisborne; Wildbore, M.J., Wellington; Southee, M.T., Porirua; Schofield, K.L., Auckland; Wise, M.L., Auckland; Dawson, T.L., Auckland; Dixon, J., Auckland; Cavanagh, G.A., Auckland; Nixon, J., Auckland; Scarborough, H.A., Auckland; Scott, T.M., Palmerston North; Smith, S.J., Invercargill; Stapleton, A.K., Christchurch; Tate, G.M., Auckland; Thomason, H.J., New Plymouth; Tipene, L.M., Kawakawa; Tiraa, A.E., Wellington; Toppin, S.J., Auckland; Twose, R.E., Auckland; Whineray, S.J., Auckland; White, F.J., Christchurch; Wickens, G.W., Auckland; Wilkie, R., Auckland; Williams, J., Auckland; Wisnewski, K.S., Hamilton; Wong, L., Auckland; Wright, K.W., Invercargill.

Applications for Associateship

Booth, E.G., New Plymouth; Connell, C.R., Auckland; Fung, S., Hong Kong; Langford, A.J., Dunedin; Lorier, M.A., Christchurch; Mace, A.C., Waipukurau.

Resignations

Beacham, J.S., Auckland; Grey, D.W., Auckland; Jackson, W., Hamilton, Knowles, H.A., Auckland; McCormick, P.D., Hamilton; Nairn, J.M., Wellington; Young, S., Christchurch.

Gone No Address

Johnstone, R.K., Palmerstone North; King, I.C., Australia.

OVERSEAS AID COMMITTEE

The Overseas Aid Committee, John Elliot, Ted Norman and Marilyn Eales recently met with Dr David Stone, Director, V.S.A. and Eileen Brindle, Recruitment and Resource Officer, V.S.A. to discuss the feasibility of short term assignments in Medical Laboratory Technology in the Pacific Area.

Over the past year the Overseas Aid Committee has been obtaining as much information as possible from people both indigenous and expatriate who are currently working in the field of Medical Technology in the Pacific area. Much of this information has been published in the N.Z.I.M.L.T. Journal under the title of "The Pacific Way". Council and N.Z.I.M.L.T. members should therefore be aware in general of where any assistance or aid needs to be directed.

The Pacific Paramedical School is serving a very useful purpose and its direction continually needs to be updated and

reassessed as more information comes back from the Pacific region. The Steering Committee of the P.P.T.C. is well aware of this and has established good liaison with the External Aid Division of the Foreign Affairs Department and with World Health Organization.

There is a need for short-term lecture tutor assignments in most areas of the Pacific and this needs to be further investigated now to find suitable ways of establishing such assignments.

(a) Requests for Aid

Clearly individuals working in laboratories see this need but any request for aid must come through the official channels usually the Health Department of the particular island concerned. Encouragement to do this is now necessary. Andrea Hall the former tutor at the P.P.T.C. on her recent assessment of returned students in the Pacific realised that the Pacific Island people are not always aware of the channels that do exist in their own country for initiating this aid.

(b) Short Term Assignments

On the surface this seems to be a simple need and an easily and attractively met request. The realities lie in the answers to the following questions:
Who goes? and for how long?
Are they suitable?
Who pays?
Who protects their current position in New Zealand?
Where does this person stay on the island he/she visits?

(c) Technologist Exchange Programme

The current economic climate in New Zealand where most of us are not over endowed with staff and therefore little flexibility in the system probably excludes such a programme at present. It is however an area which should be considered as there could be definite advantages in establishing a two-way system at some future date. The discussion with V.S.A. centres around the questions raised in (a) and (b) above. At present there are no definitive answers to these questions. It would appear however that some short term assignments in conjunction with V.S.A. may be possible. The Overseas Aid Committee and V.S.A. are currently looking into the practical and financial aspects of such assignments.

Council have accepted the recommendation of the Overseas Aid Committee that all students at the P.P.T.C. on completion of their course receive a complimentary copy of the textbook "Medical Laboratory Manual for Tropical Countries", Volume II by Monica Cheesbrough, F.I.M.L.S. Tech. R.M.S.

Branch News

Auckland Branch

These are the members for the 1985 committee of the Auckland Branch of the N.Z.I.M.L.T.

Chairman:	Mr Ron Law	— Biochemistry Tutor, Wallace Laboratory, Auckland Hospital.
Secretary:	Mr Ian Guild	— Microbiology Laboratory, Green Lane Hospital.
Treasurer:	Mr Peter Wyatt	— Biochemistry Laboratory, National Womens' Hospital.

Ordinary Committee Members:
Ms Karen Rogers
Mr Steven Henry

NZIMLT Library

The following Journals have been recently received. They are available for loan from The Librarian, Mr J. Lucas, Haematology Dept. Dunedin Hospital.

Medical Laboratory Sciences Vol 41, 3

1. Quality assurance in haematology and blood coagulation testing

Original Articles

2. A whole blood control for Coulter™ electronic particle counters
3. A rapid-return regional quality assurance scheme for coagulation
4. Effect of hydroxyethylpiperazine N-2 ethane sulphonic acid (HEPES) on the thrombin clotting time and fibrin polymerisation
5. A direct enzyme-linked antiglobulin test for detection of red cell autoantibodies in auto-immune haemolytic anaemia
6. Immunoelectronmicroscopy: immunogold and immuno-peroxidase compared using a new post-embedding system
7. Lymphocyte surface marker techniques in the routine pathology laboratory

Annotations

8. Clinical aspects of steroid receptor assays
9. Antithrombin III screening: necessity or extravagance
10. Properties of some of the newer cephalosporins
11. Localization of tumours using radiolabelled antibodies

Short Communications

12. An enclosed fixative preparation system
13. Demonstration of eosinophil polymorph granules in sections of paraffin- and meth-acrylate-embedded tissue: a new method
14. A simple questionnaire for use in computerizing bone marrow aspirate reports
15. External quality assessment: the effect and implications of favourable treatment of EQA samples
16. Determination of Cefotaxime in plasma by high pressure liquid chromatography
17. Microchemotaxis: a procedure for embedding and ultrastructural study of migrating leukocytes within filter membranes

Australian Journal of Medical Laboratory Science Vol 5, 2

1. Human Complement Component C4 and its Relationship to the Major Histocompatability Complex — Review
2. Rubella Enzyme Immunoassay — Can Standardization be Achieved?
3. Electrophoretic Mobility Study on the effect of time on the Enzymatic Treatment of Human Erythrocytes
4. Evaluation of the AO Unistat Bilirubinometer
5. Comparative Epidemiology of Melanoma in Three Australian States
6. Cross-reactivity in Immunoperoxidase Prostate Specific Antigen

Australian Journal of Medical Laboratory Science Vol 5, 4

1. Autologous Transfusion — Review
2. Haemoglobin N (Baltimore) in a Timorese Lady (Case Study)
3. Job Design and Job Satisfaction of Clinical Pathology Laboratory Workers: An Australian Perspective
4. A Calcium Phosphate Co-sedimentation Method for the Preparation of Haemoglobin-free Human Erythrocyte Stroma
5. Salicylate Levels in Blood Donations collected in Brisbane

Journal of Medical Technology Vol 1, 5

Focus on Computer Application

1. Computer Graphic Representation of Multivariate Laboratory Data
2. Computer Graphics to Assess Trends in Glomerular Filtration Rate from Sequential Serum Creatinine Values
3. Using Expert Systems for Interpretive Reporting
4. Medical Information Systems: Current Aspects and Implication
5. Protocol for the Use of Lewis-Neutralized Serum in Prenatal Antibody Screening
6. A Rate Nephrolometric Inhibition Immunoassay for Quinidine and its Active Metabolites

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Focus on Immunology/Immunohaematology

1. Clinical Chemistry Profile of Hepatitis B
2. Clinical Serological Aspects of Hepatitis B
3. Immunoprophylaxis: Towards Control of Hepatitis B
4. Component Therapy — Current Concepts
5. *Staph. Saprophyticus* — Literature Review
6. Cost Containment in the Blood Bank: Elimination of Unnecessary Serological Testing

Canadian Journal of Medical Technology Vol 46, 1

1. The Immunological and Biochemical Characterization of Leukaemia-associated Antigens
2. Demonstration of Juxta-glomerular Cells in Methacrylate Embedded Tissue
3. Inactivation of Creatinine Kinase by Light

Canadian Journal of Medical Technology Vol 46, 2

1. Comparison of Three Microchromatographic Methods for the Quantitation of HbA_2
2. Tube Holder Attachment for Inverted Microscopes

Laboratory Medicine Vol 15, 6

1. Haematology Instruments for Clinical Laboratories
2. Blood Transfusion in Autoimmune Haemolytic Anaemia
3. Immunocytochemistry Markers in E.M.: Peroxidase and Colloidal Gold
4. May-Grunwald Giemsa Stain for Plastic Embedded Bone Marrow Specimens
5. Isolation of *Haemophilus parainfluenzae* in a Case of Endocarditis
6. One Low Litmus Milk Test for the Identification of *Strept. faecalis*

Laboratory Medicine Vol 15, 7

1. Specific and Not-so-Specific Histiocytes in Bone Marrow
2. Decreased T-Helper/Suppressor Ratios in Homosexual Men — A Non Specific Finding
3. Evaluation of the Para-Pak (C & S) Stool Transport Medium
4. Correction for the Effects of Lipemia on Ortho ELT — 8 results

Laboratory Medicine Vol 15, 8

1. Consideration of Automation in Clinical Microbiology
2. Bactec Automated Blood Cultures
3. Detection of Bacteremia by Automated Methods
4. Gas-Liquid Chromatography in the Clinical Microbiology Laboratory

Laboratory Medicine Vol 15, 9

1. Transfusion and Disease Transmission — Risks and Realities of AIDS and HTLV-III
2. Dry Chemistry Reagent Systems
3. Immunosuppressive Drugs and Leukocyte Enumeration in Renal Transplant Recipients
4. Haemoglobin Raleigh and Glycosylated Haemoglobin
5. New Approach to Antibodies Directed at High Frequency Kell and Cartwright Antigens using D77 - Modified RBC.

Answers to Section V: Part 3

1. a) 2.8-4.8
   b) 7.1-9.1
   c) 8.7-10.7
   d) 1.4-3.4, 8.8-10.8
   e) 1.1-3.1, 6.2-8.2, 11.3-13.3
   f) 2.1-7.4
   g) 2.0-5.4
2. a) pH = 1.0 before dilution, pH = 2.0 after dilution
   b) pH = 4.8 before dilution, pH = 4.8 after dilution — pH is dependent upon the ratio of [salt]/[acid] and not on the absolute concentration.
3. pH change of 1.0 mol/L acetate buffer is from 4.8 to 4.8 (no appreciable change).
   pH change of 0.1 mol/L acetate buffer is from 4.8 to 4.6.
4. a) 100 mL of mol/L hydrochloric acid + 200 mL of 1 mol/L sodium acetate gives a 0.67 mol/L acetate buffer pH 4.8.
   b) 150 mL of 1 mol/L acetic acid + 150 mL of 1 mol/L sodium acetate gives a 1.0 mol/L acetate buffer pH 4.8.
   c) 200 mL of mol/L acetic acid + 100 mL of 1 mol/L sodium hydroxide gives a 0.67 mol/L acetate buffer pH 4.8.
5. a) pH = 4.3, b) pH = 4.8, c) pH = 5.3
6. a) Na^+ = 0.15 mol/L, b) Cl^- = 0.1 mol/L, c) acetate =0.05 mol/L, d) $[OH^-] = 6.31 \times 10^{-10}$, e) $[H^+] = 1.58 \times 10^{-5}$
7. a) Tris = 0.02 mol/L, b) Tris-HCl = 0.08 mol/L, c) 79 mL
8. a) Cl^- = 0.08 mol/L, b) Tris 0.04 mol/L, c) Tris-H^+ = 0.08 mol/L, d) $[H^+] = 1.66 \times 10^{-8}$ mol/L
9. a) Na^+ = 0.05 mol/L, b) butyrate ions = 0.05 mol/L, c) pH = 5.00
10. $[H^+] = 5.0 \times 10^{-8}$ mol/L, no
11. Urate ([Urate$^-$] = 3000 × [Uric Acid])
12. Succinate ([Succinate^{--}] = 58 × [Hydrogen Succinate$^-$] and [Hydrogen Succinate$^-$] = 1500 × [Succinic Acid])
13. a) pH = 2.2, b) pH = 10.0
14. a) $[H_2T]$ = 0.012 mol/L, b) $[HT^-]$ = 0.069 mol/L, c) $[T^{--}]$ = 0.019 mol/L, d) 107 mL
15. pH = 4.8
16. pH = 8.46 ($[B^-]$ = 0.287 mol/L, [BH] = 0.026 mol/L, $[TH^+]$ = 0.056 mol/L, [T] = 0.134 mol/L.

MLTB News

Medical Laboratory Technologists' Board Newsletter March — 1985

The Medical Laboratory Technologists' Board met in Wellington on the 27th February, 1985. Although a number of topics were discussed, this meeting was mainly concerned with examining the reports from the previous years examinations and making recommendations for the conduct of examinations for the current year. There are a number of matters in this regard which are brought to the attention of laboratories.

Examination Reports

The Board for a number of years has asked its examiners to prepare comprehensive reports on the examinations and in addition to this prepare reports on a confidential basis on "failed" candidates. The Board would request that these reports are used to full advantage — that failed candidates have their reports discussed with them and that the general report on the examination is circulated to all those who are involved in preparing candidates for examinations as well as the intending candidates. This year a number of requests were received from last years candidates to have their written papers released to them under the Official Information Act. The Board has decided that such papers will be released on personal application from candidates. This year applications will be received and actioned up until July but in following years applications must be received before the Board's February meeting. After the February meeting examination papers will be destroyed as per our present policy. In line with the decision to allow scripts to be released the Board will no longer require examiners to prepare reports on failed candidates as there will be an opportunity for tutors and charge technologists to review a failed candidates paper if the candidate so desires and makes personal application for the paper.

Examination Fees

At the present time the fees are the same as they were last year. However the Board has been informed by the Department that a review of fees is taking place and if these are gazetted before the closing date of the examination, the Board will be required to pass on to candidates, the additional fees that may be incurred.

Date of Examinations

There has been concern over the years at the sometimes rather protracted gap between theory examinations and practical examinations. Obviously the Board needs to have regard to the fact that practicals are conducted in home laboratories and for this reason all practicals cannot be held at the same time. However in an attempt to reduce the gap between the written examination and the practical examination, the following timetable will be observed this year:

THEORY EXAMINATIONS in the week commencing 14th October.

PRACTICAL EXAMINATIONS for Haematology, Virology and Histology: in the week commencing 21st October.

PRACTICAL EXAMINATIONS for Clinical Chemistry, Immunohaematology and Immunology: in the week commencing the 28th October.

PRACTICAL EXAMINATIONS for Microbiology, Cytology and Nuclear Medicine: in the week commencing 4th November.

The Cytogenetic examinations will follow their normal practice with the extended practical.

Examination Papers

In a continuing effort to ensure that papers are printed correctly

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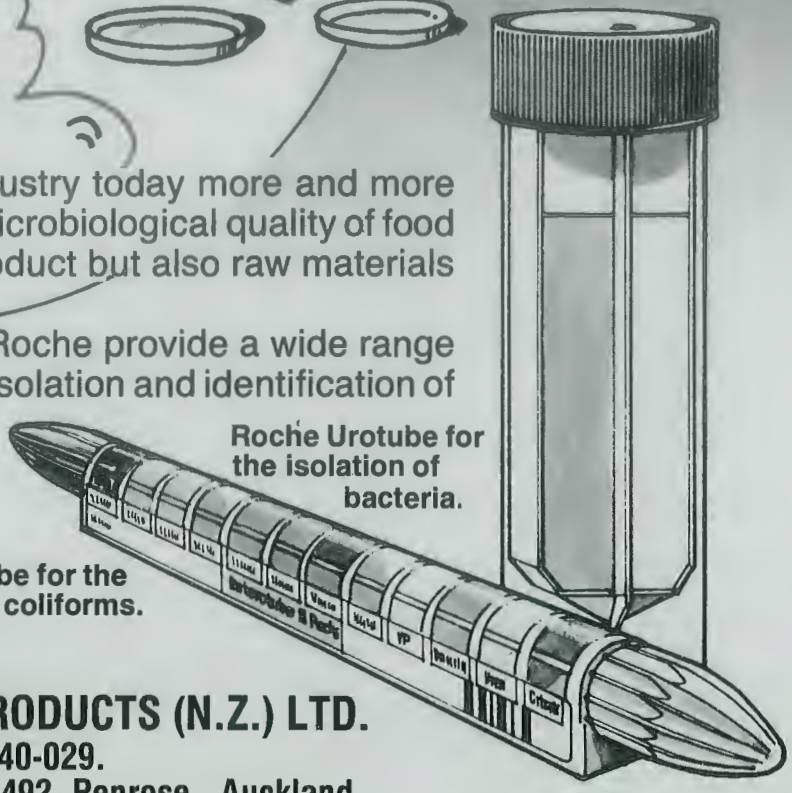

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and are received by examination centres in good time, the final papers will be printed from the copy of the examination sent to the Department by the senior examiner. Papers will not be re-typed in the Department and examiners in preparing papers should bear in mind the quality of print and the layout of the paper that they are forwarding to the Department as this copy will be used for photocopying.

Examiners Panel

The N.Z.I.M.L.T., and the N.Z.S.O.P. are to be asked to forward the names of potential examiners for the Boards' examinations.

Computer Assistance

At the present time the Boards' method of notifying candidates is a rather laborious method with a large human input with the inherent potential for the occasional error in the reporting of examination results and the Board has resolved that it will once again request the Department to make some computer assistance available.

Closing Dates For Examination

Laboratory charges, tutors and candidates are reminded that the closing date for exam applications is **July 15th** and that the Boards' policy is not to accept late applications. There is no reason why candidates should not send their examination entries early in the year and in fact, the Secretary would be prepared to accept entries from now onwards. The examination entry application need not necessarily contain the fees at this stage.

Desmond J. Philip,
Chairman

New Product and Services

COMPUTERISED SYSTEM TAKES DRUDGERY OUT OF MICROBIOLOGY

A new computerised microbiology laboratory system produces results in hours that used to take several days using traditional manual methods. The Vitek AutoMicrobic System (AMS), a product of McDonnell Douglas Health Systems, also produces results that the company says are consistently more accurate than manual tests. Operation of the system is very simple. Microbial specimens are placed in a test card which is then inserted into the machine. The system then examines the card and automatically prints out a full report based on the results of 30 tests which it has carried out on the specimens. These tests traditionally take about two or three days, but with the AMS a report can be produced as early as three hours. The printed report first identifies the organisms present in the test card and then suggest the most effective methods of treatment. This speedy analysis means that the offending organism can quickly be identified and treated, resulting in shorter length of stay for the patient. It also prevents use of the "scattergun" approach — which, in the past, may have been necessary — or giving the patient a large dose of antibiotics while doctors await the outcome of tests. This practice, to which, admittedly, in some cases there has been little alternative, has encouraged the spread of Golden Staph and all its attendant problems and costs — in some instances, even deaths. The Vitek AMS also liberates microbiologists from the repetitive tasks normally performed in the lab and gives them the time to do tests which in many cases are presently handled by reference laboratories.

According to Bill Antonopoulos, Australian Marketing and Sales Representative for Viket, "Microbiology is the last field of pathology to automate, and Vitek is the undoubted pioneer of the technology that enables the system to do what it does. The test cell, which is no bigger than a playing card, consists of 30 micro wells which are each tested for a specific biological reaction." Antonopoulos noted that at present, a specimen must be analysed and the organisms it contains separated in order to identify the causative microbe. The organisms must be allowed to grow and then be examined hourly for growth patterns and test reactions. "There can be many organisms in the specimen and tests are necessary to identify the pathogen. These tests traditionally take three or four days," he said. "But with the Vitek AMS you can load direct specimens, such as urine and stool samples, and get an identification and enumeration of each organism in a specimen, which may contain a wide range of organisms in as little as three hours. The system then prints out a report." And all this for less than $1 per specimen. Antonopoulos said that the system offered advantages in cost and efficiency to hospitals of all sizes. There are systems to suit the smallest or largest institution. "The Vitek system is the only one in the world which can identify in four hours difficult organisms, such as anerobes, Neisseria and Haemophilus, without the requirement of anerobic conditions," he said. "And this is a major breakthrough in microbiology."

Software for the system is upgraded every three months, and this includes new test capabilities. These upgrades are supplied free.

For further information: Judy Pehrson (04) 724-190 or **circle 4 on the Readers Reply Card.**

IMPROVED COST CONTAINMENT FOR COBAS BIO ANALYSER USERS

Laboratories using the Cobas Bio analyser now have a cost saving source of consumables with the introduction of Elkay's new sample microtubes.

Formulated and precision molded by Elkay to meet the equipment manufacturers specifications, the new Cobas Bio type microtubes have two extra features for the benefit of users of this analyser. The internal configuration of the microtube has been designed to reduce sample volume deadspace by up to 20%. This is particularly helpful when pediatric samples are being analysed. Elkay microtubes are also available in 5 different colours, including natural, so that samples such as STATS or toxins can be colour coded by sample holder.

The new sample microtubes are an addition to the Elkay line of consumables for Cobas Bio users which includes thermal printer paper. All of these products are listed at prices significantly lower than those published by the original equipment manufacturer.

For further information contact Medic DDS, P.O. Box 205, Wellington or **circle 3 on the Readers Reply Card.**

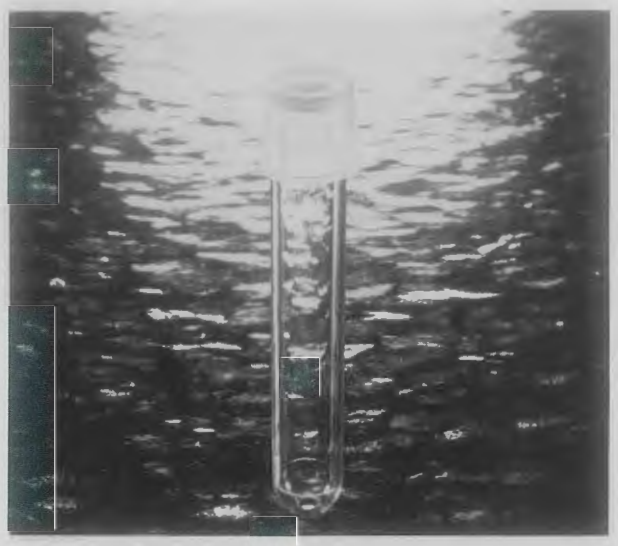
WOMEN SCIENTISTS OPEN BIOTECH PLANT

A new manufacturing venture in Auckland aims to make New Zealand substantially self-sufficient in antisera and other biological products used by medical and veterinary research establishments, and diagnostic laboratories. Immuno-Chemical Products Ltd (ICP) has begun marketing a range of animal sera and blood proteins, including 32 antisera, which are being made at a high-technology laboratory in Parnell. All the products have been imported up till now.

The company is headed by two women, Dr Rosemary Sharpin PhD. and Mrs Maxine Simmons MSc. Both are experienced medical research scientists. "We are combining the use of New Zealand scientific resources and international technology to add value to New Zealand's renewable raw materials. In many cases these raw

materials have previously been wasted," said Dr Sharpin. She added that, apart from generating exports and saving overseas funds by import substitution, the new business will eliminate long delays commonly experienced in obtaining the products from overseas. It will also decrease the risk of exotic disease being inadvertently imported in these products. "Research and development of new products from New Zealand resources will be a significant part of our work and will start in earnest once the business is fully established," she said.

The initial range of products includes antisera to human and animal blood proteins, cell separation media, purified albumins and complement. Dr Sharpin said ICP is confident of gaining orders overseas and is already negotiating an export contract.



NEW STERILE CULTURE TUBES FOR ALL LABORATORY NEEDS

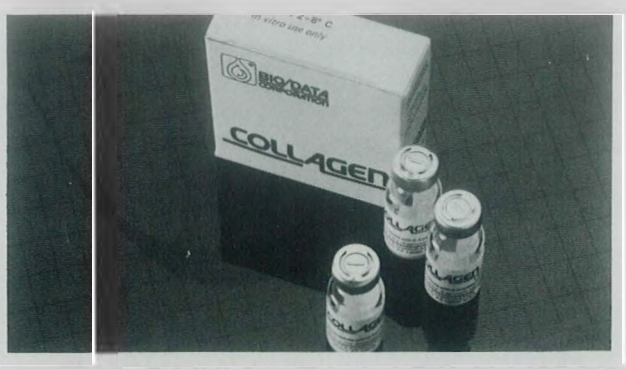
Elkay's new line of disposable sterile culture tubes has been designed to comprehensively meet the diversified needs of today's laboratory technologist.

There are nineteen different configurations in the new range in sizes from 10 x 75 mm. through 16 x 150 mm. These include six choices of tube capacity from 3.5 ml through 20 ml and two types of leaktite snap-on and screwcaps. The tubes are precision molded by Elkay in polystyrene for optical clarity and in polypropylene for durability.

All of the products are sterilized in accordance with U.S. Pharmacopeia Volume XX and conveniently packaged in stackable trays containing 25 and 125 tubes or individually wrapped.

In common with all Elkay disposables, the new sterile culture tubes are guaranteed in writing to equal or outperform any comparable products.

For further information contact Medic DDS, P.O. Box 205, Wellington or **circle 1 on the Readers Reply Card.**



NEW LYOPHILIZED COLLAGEN FOR PLATELET AGGREGATION

Lyophilized Collagen, prepared specifically for use in platelet function studies, is now available from Bio/Data Corporation. The reagent is produced from calf skin which consists primarily of Collagen Type III found to be a particularly effective platelet aggregant. A new manufacturing process facilitates reconstitution of the Lyophilized Collagen while enhancing storage and shelf stability.

Collagen is recognized as a fundamental aggregation reagent for the identification and evaluation of qualitative platelet function abnormalities. When collagen is added to platelet rich plasma, the platelets adhere to the collagen. Following this adhesion, normal platelets will change their shape, release stored nucleotides, and aggregate. This aggregation is observed with a platelet aggregometer. An abnormal response to collagen could be indicative of recent aspirin ingestion or of an inherited platelet dysfunction such as Storage Pool Disease or Glanzmann's Thrombasthenia.

Collagen is supplied in 10 determinations vials at a concentration of 1.9mg/ml. Each package contains three vials of stable, readily soluble, lyophilized reagent. Following reconstitution with distilled water, the material may be stored for up to 30 days at 2-8°C. Collagen may be used with any platelet aggregation instrument.

For further information on Collagen, Catalog No. 101562, contact Bio/Data Corporation, 3615 Davisville Road, Hatboro, PA 19040, USA; 215/441-4000; Telex: 834482 or**circle 8 on the Readers Reply Card.**

NEW DISPOSABLE FOR LARGER VOLUME CENTRIFUGING

Elkay has introduced a disposable screwcap tube for larger volume centrifugation. Precision molded by Elkay in biologically inert polypropylene, the 50 ml capacity tube is available sterile and non-sterile.

The tube has bold graduations in 2.5 ml increments from 2.5 ml to 10 ml and in 5 ml increments to 50 ml to make sample volume easy to assess.

A leaktite threaded cap insures a positive seal so the tubes can be used for collecting, processing and transporting specimens as well as centrifugation.

The sterile tube is gamma radiated in accordance with U.S. Pharmacopeia Volume XX and uniquely packaged in peel apart, stackable trays containing 25 tubes each.

With the introduction of 50 ml tubes Elkay now offers laboratories a comprehensive line of sterile and non-sterile centrifuge tubes at competitive prices.

For further information contact Medic DDS, P.O. Box 205, Wellington or **circle 9 on the Readers Reply Card.**

NEW DIMENSION IN ELECTRON MICROSCOPY

Carl Zeiss are proud to introduce a new dimension in electron microscopy the revolutionary analytical TEM, EM 902. Including a fully integrated electron energy loss spectrometer and exclusive capability of electron spectroscopic imaging ESI, the new TEM enables for the first time in a series produced EM to analyse and image light elements improve image quality and contrast in thick sections or thin unstained specimens and to obtain energy filtered diffraction patterns.

These are just some of the most important characteristics of the EM 902 and of course conventional TEM is always possible.

The EM 902 represents the key in decoding the abundance of information and secrets hidden in the electron beam after interaction with the specimen.

For further information please contact: Carl Zeiss Pty. Ltd., 4th Floor, Mayfair Chambers, The Terrace, Wellington or **circle 7 on the Readers Reply Card.**

BECKMAN INTRODUCES IMMUNOFIXATION ELECTROPHORESIS FOR ABNORMAL PROTEIN IDENTIFICATION

An Immunofixation Electrophoresis (IFE) assay kit has been introduced in the Paragon™ Electrophoresis product line by Beckman Instruments, Inc.

Immunofixation electrophoresis has been simplified for routine clinical use from research laboratory techniques. The new Paragon IFE test provides a fast, sensitive and easy to read method for diagnostic identification of monoclonal gammapathies, immunoglobulin deficiencies and other immunoglobulin anomalies.

IFE is a procedure which combines the principles of protein electrophoresis and immunoprecipitation. With IFE, a specimen (serum, plasma, cerebrospinal fluid or urine) is first electrophoresed in multiple positions on an agarose gel. Following electrophoretic separation of the proteins, monospecific antisera to IgG, IgA, IgM, kappa and lambda are applied directly onto the gel surface. A protein precipitating solution is applied in one position along with the antisera. The immunofixation reaction occurs during a 30 minute incubation period. The resulting antigen antibody complexes become trapped in the gel pore structure. Uncomplexed serum proteins and excess antisera are then removed from the gel. The remaining immunoprecipitin complexes and the precipitated protein reference pattern are stained for interpretation. Total test time is approximately two hours.

The Beckman Paragon IFE procedure offers the following technological advances for adapting IFE for routine clinical use: (1) The procedure has been simplified to require minimum sample dilutions and needs no special equipment such as a cooling device. (2) A unique template methodology is utilized for both specimen and antisera application. This eliminates the need for antisera saturated paper strips and allows for direct reaction of antigen and antibody, thereby increasing sensitivity and dynamic range. (3) The antisera provided are fractionated and colored and result in no background staining which enhances interpretation and sensitivity. (4) An integral serum protein reference pattern is provided on each gel which requires no additional processing.

Paragon IFE is one of nine electrophoresis test assay kits available from Beckman. Other kits are Acid Hemoglobin, Hemoglobin, Serum Protein Electrophoresis (SPE and high resolution SPE-II), Lactic Dehydrogenase Isoenzyme (LD), Creatine Kinase Isoenzyme (CK), Immunoelectrophoresis (IEP) and Lipoprotein. Also, available from Beckman are electrophoresis controls for SPE (normal and abnormal), LD, CK and Hemoglobin and complete test processing equipment—power supply, incubator, dryer, we processor, gel frames, applicators and pipet tips.

For more information, contact Alphatech Ltd phone 770-392 Auckland or **circle 10 on Readers Reply Card.**

RISTOCETIN FOR CLASSIFICATION OF VON WILLEBRAND VARIANTS

Hatboro — PA, Aggrecetin®, high purity ristocetin, from Bio/Data Corporation is the only ristocetin available in a variety of concentrations for analyses of von Willebrand Syndrome variants.

One such analysis involves the evaluation of platelet aggregation with various concentrations of ristocetin. Platelets from a patient with von Willebrand subtype IIB characteristically aggregate with lower concentration of ristocetin than required to aggregate platelets from normal subjects.

Another analysis also requiring ristocetin is the assay of the von Willebrand Factor (ristocetin cofactor). Quantitation of this plasma factor is an essential element in the classification scheme of von Willebrand subtypes. The concentration of ristocetin required for this assay is generally lower than ristocetin used in platelet aggregation studies.

For the quantification and qualification of von Willebrand variants, only AggRecetin® is available in 15 milligram vial sizes with three diluent variations: 1.5mg/ml, 1.2mg/ml, and 1.0-1.5mg/ml. Bio/Data Corporation also provides a 100 milligram size for added economy.

For more information on AggRecetin® and other Bio/Data Corporation reagents, please contact Bio/Data Corporation, 3615 Davisville Road, Hatboro, PA 19040, 215/441-400 or **circle 2 on the Readers Reply Card.**

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Microbiology

A registered medical technologist is required for the position of second in charge of the Microbiology Department of a Lower Hutt private Medical Laboratory.

Preference will be given to applicants who have A-level Microbiology.

For appropriate experience starting salary would be on the graded scale.

Apply — Personnel, Technologist Valley Diagnostics, P.O. Box 30044, Lower Hutt.

CYTOLOGY TECHNICIAN

We have a vacancy for a qualified or partially qualified technician for our Cytology Department.

It is envisaged that this person would assist with the routine screening and general departmental work.

The department is well equipped with modern computers to help with patient cumulative records.

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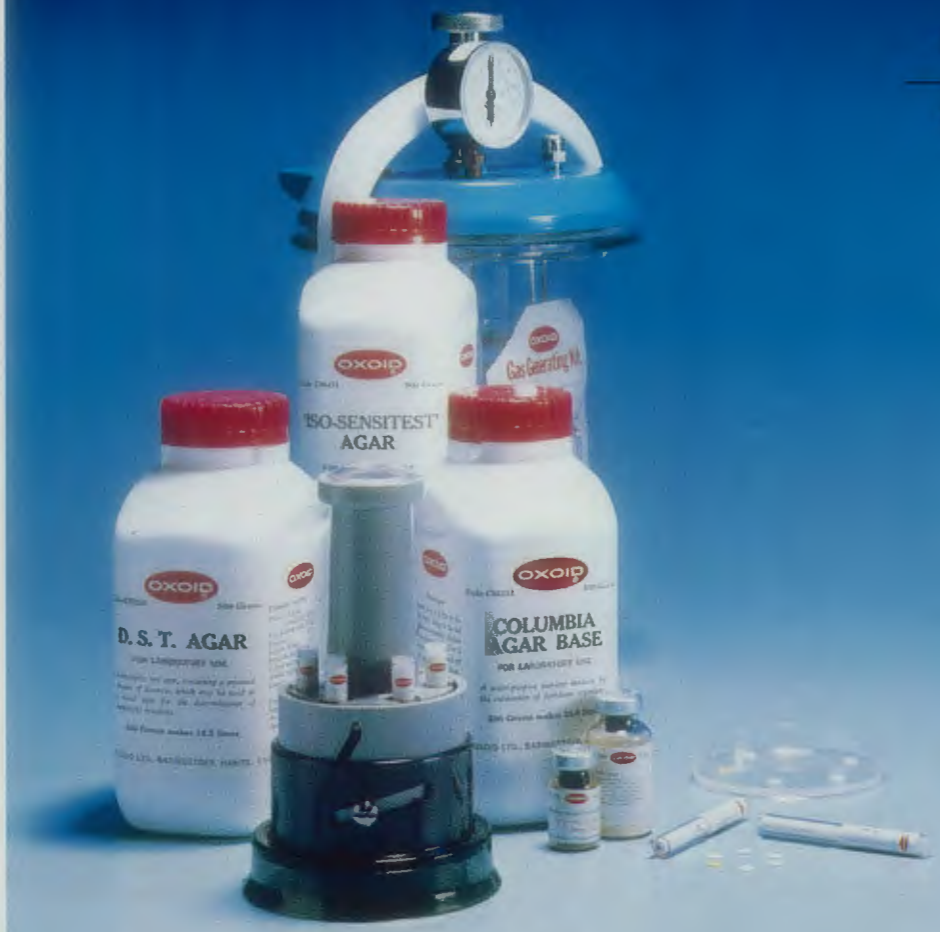

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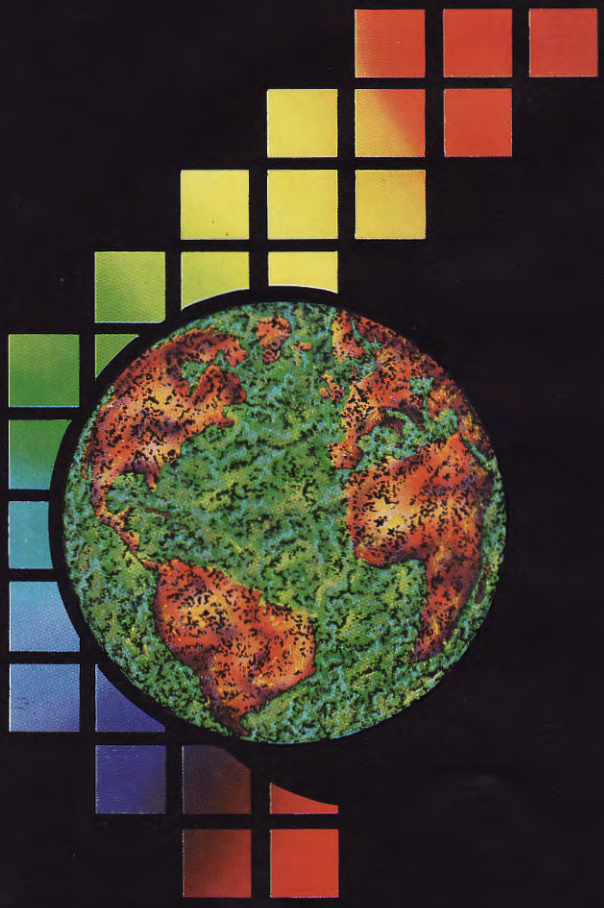

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So anywhere in the world microscopists can be sure of identical testing parameters and feel confident of repeating their diagnoses with the same degree of reliability and consistency. And getting the best possible results from their microscopes.

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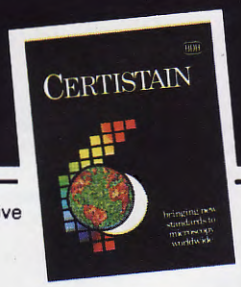
The Certistain range. Initially 30 stains. Followed by a further 30. Ultimate total — 200 stains.

For our FREE and fully comprehensive handbook on the Certistain range 'please send the coupon to BDH Chemicals Ltd'.



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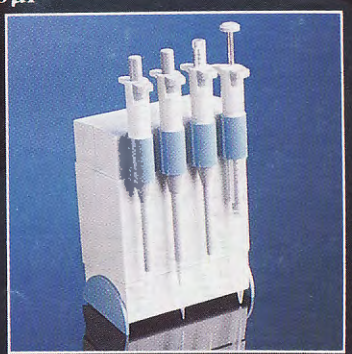
831 0,2 to 5ml with PP tip, 0,2 and 0,5 ml increments
0,2 to 2ml with glass Pasteur pipette, 0,2 and 0,5 ml increments



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