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From Vol. 36 No. 1 all papers published will be in the form known as "Vancouver Style" or Uniform Requirements for Manuscripts submitted to Biomedical Journals. Full details may be found in the New Zealand Journal of Medical Laboratory Technology, Vol. 36, No. 4, page 90 to 109 or from the Editor.

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enlarge on today.

The governments of our two countries are on the verge of signing an agreement called Closer Economic Relations commonly abbreviated, at least in our papers, to C.E.R. This is an agreement to replace N.A.F.T.A. which has now more than run its useful course and no longer caters for the trade requirements between our countries. C.E.R., which has been years in the planning, will provide a reduction in tariffs, import licencing and trade barriers generally. As I understand it, it will be a long operating agreement with no definite date set for its end, and it will allow our countries to produce and export those things they are good at, with a consequent improvement to our economies. It seems highly likely that this agreement is going to operate from January next year with a review planned for 1988. As with all these things there will almost certainly be some teething problems but given the will to work it will be beneficial to all parties in the long term.

Will this new agreement have any effect on medical science and technology in our two countries? Apart from some obvious pricing changes in equipment and supplies I am not aware of any specific way in which the Closer Economic Relations agreement is going to affect us directly in our Institutes. However, to fit in with my fable I would like to take the title C.E.R. and consider some aspects with the letter 'E' standing for something other than 'Economic'.

From the various parts of this disjointed speech I would like to draw several salient points to our consideration.

Firstly — Australia and New Zealand have come from a single common source and as such have much in common. This is true not only geologically as we have seen, but also culturally, socially and historically.

Second — As a continuing geological feature the plates on which our land exists actively meet and merge together. The result of this as we saw is often exhibited in volcanic activity. This week, for us, is a meeting of the 'plates' of medical technology within our countries. Just as the geological interaction results in demonstrable activity I trust that this meeting of our Institutes will result in some positive action. Volcanic action can vary. We have a mountain in the North Island which rumbles and grumbles away and sends out great plumes of steam and smoke but very seldom gets to the stage where something earth-shattering happens. It is possible that like Ngauruhoe in the North this week could produce a lot of ephemeral smoke and steam but nothing more lasting. We, as the individuals who make up our respective bodies, are the ones to ensure that does not happen.

Volcanoes can be very destructive but they can also be very constructive. One reads with interest of the fertilizing and renewing effect of Mt. Etna's eruptions which at first appeared to be wholly, totally, and solely destructive. My hope is that from the meeting of our two Institutes action will occur. We may have to be prepared to see some of that activity as destructive — some of our own pet ideas and cherished notions may have to go. If, however, it is solely destructive then it will have been a waste of time. If, like Mt. Etna's fallout it is regenerative, enriching and productive then time and effort will have been well spent.

I am no longer on the Council of the New Zealand Institute and when one does not have to be intimately involved in the hard work of change it is easy to make suggestions. Nevertheless I would like to propose today some areas in which I feel our Institutes should be involved which brings us to the final point in our parable.

Third — C.E.R. — Closer Economic Relations. With the licence I asked you to indulgently grant me I would first like to change that C.E.R. to **Closer Extra-Mural Relations.**

At the recent Congress of the I.A.M.L.T. held in Amsterdam the delegates resolved that the Association should be involved as a validating body for qualifying examinations in third world countries who requested such validation. Part of the Constitution of the International body states that as one of its objectives the Association will 'catalogue the training standards in different countries, in order to prescribe the minimum standards of training (in collaboration with W.H.O.), and to raise the standard of training of medical laboratory technologists.' Mindful of its responsibilities in this area the Congress resolved to establish a panel of experts to write rules for examinations, establish administrative machinery for validating such examinations and inaugurate as soon as possible a pilot scheme with some governments and governmental agencies. The delegates very firmly decided that the I.A.M.L.T. should be involved in such schemes only after they had approached the representatives of medical laboratory technology within that country. This, I feel certain, will be the strength of what I see as a positive and innovative step by the International body. The technologists in the area must be involved. They will know better than any the needs and requirements of the area in regard to their discipline — assessed of course with guidance from advisors such as W.H.O. The International body will therefore work in close relationship with W.H.O. It is most likely that first areas to be examined will be in the Middle East — Oman and Jordan. The pilot scheme will no doubt take some time to work through to a final and fruitful conclusion.

I would like to propose that we — that is Australia and New Zealand — have a very real responsibility to work in a similar way in the South Pacific area. I am certainly not advocating a split in the International Association but I do see that there must be regional areas of responsibility for the stronger, technologically well-developed countries. This area of responsibility for us is clearly indicated — although I am not certain how far we push the boundaries. We have a responsibility to act and help in these areas — a responsibility I believe we neglect to our own detriment. President Neal in his report to the annual meeting of the I.A.M.L.T. said 'our history reveals that medical laboratory services contributed to the improvement in health care. Responsible attitudes and dedication will enable us to take a leading role in meeting new challenges in the future'.

Already, within both of our countries, we have seen a willingness to help. Western Australia through its Institute of Technology has a scheme for educating students from Papua New Guinea. In Wellington we have the Pacific Para-Medical Training Centre which exists with the help of a number of agencies including our own Institute. These are positive steps and need our continued backing. But I would like to challenge us to ask if we are doing enough.

How about some extensions to our training schemes to include those with needs in the Pacific area? How about some exchanges to allow people from the Islands to work in our laboratories and we in theirs — to the mutual benefit of both sides? How about some tapes and slides specifically catering for needs as they are assessed? How about satellite links to allow for training, information-dissemination, problem solving etc. How about — dare I suggest it? — some seminars at Congresses such as these on the problems of 'clapped out' flame photometers as well as the esoteric computer controlled multi channels. Hospital authorities, health departments and governments must be made to recognise their own responsibilities in these areas. I don't know whether these ideas are within the proverbial bull's roar of the requirements. My conviction is that there is a need — one I have seen demonstrated and so have you. What is needed? Today we have here a representative from W.H.O. Manila. Here is an agency with whom we can work — an agency who will assess, in conjunction with technologists, the needs within the Pacific area. 'A do-gooder' do I hear someone mutter? Well if that is the label for people who are concerned for those who are less well-catered for and wish to help them, then I am happy to wear the label — and so too will be our Institutes as a whole. It won't be a one way traffic if someone has to see pluses in anything they do. I can't speak for Australia but here in New Zealand we are needing members of the Health team who have had experience working with people from the Islands — understanding them — their problems and their illnesses.

Let me turn very briefly in conclusion to one other C.E.R. — Closer Educational Relations. Let me say right at the outset that I recognise and support the need for all countries to develop education schemes that fit their own particular requirements. That I would never want to change although I believe there is room to introduce a post qualification examination at the top level which will allow evaluation between countries — a Fellowship of the I.A.M.L.T. or an associated College. Right now, though, I would like to invite the Councils of our Institutes to work together to establish an acceptance of qualifications — not an equivalence but a recognition which will allow portability. It seems quite incredible to me that with two countries who have so much in common we have difficulty in doing just that. In New Zealand we have problems with Registration regulations — Australia has problems in other areas. Let's not look at the problems — sigh — and dismiss reciprocal acceptance as impossible, let's work towards it.

C.E.R. — What price Medical Science? The price to achieve my nominated C.E.R.'s will be hard work, a willingness to perhaps compromise, maybe a swallowing of pride and a giving away of some warm, comfortable but introspective notions. The pay-off could be that when we meet again, as I trust we will, then this Congress will be, by representation, by participation and by involvement, truly — THE SOUTH PACIFIC CONGRESS.

Assay of Serum Vitamin B_{12} and Folate — A Comparison of Methods

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Based on a paper presented at the 1982 South Pacific Congress in Medical Laboratory Technology, Christchurch

Abstract

Two commercially available radioisotope assay kits (Becton Dickinson Simultrac and Diagnostic Products Corporation Dual Count) were compared with the Euglena and Lactobacillus assays for the determination of serum vitamin B_{12} and folate.

Blood samples were selected for analysis from hospitalised patients in whom the majority had a serum vitamin B_{12} level of less than 200 pmol/l as determined by the Euglena assay and where relevant haematological and clinical data would be obtained. Ninety-six samples were analysed.

For vitamin B_{12} the correlation between the Euglena assay and radioisotope assays were: r = 0.8331 DPC, 0.8324 Simultrac with the Euglena assay tending to give lower values. Patients on antibiotics (mainly Bactrim) were excluded from this statistical analysis. There was good correlation between the folate assays; r = 0.7696 DPC, r = 0.8066 Simultrac. The *Lactobacillus casei* assay tended to give higher values.

No values in the normal range were obtained with evidence of megaloblastic change although both microbiological and radioisotopic assays produced a small number of sub-normal values in the absence of expected haematological changes.

Key Words:
Vitamin B_{12} and Folate, Radioisotope Assay.

Introduction

Over the last decade in New Zealand an increasing number of commercially available radioisotope kits for the assay of vitamin B_{12} and folate have become widely used. These may be the single assay or double assay type in which vitamin B_{12} and folate result are obtained from a single serum aliquot. Furthermore, frequent modifications are being made to these kits which presents difficulties in determining the reliability of each methodology and in assessing the differences in results obtained as a result of each modification. This aspect is a particular problem for smaller laboratories who wish to begin vitamin B_{12} and folates where no previous assays were performed.

The most frequently used reference methods have been the long established microbiological assays.[1,2] Earlier work demonstrates entirely satisfactory sensitivity when employing microbiological assays, particularly for vitamin B_{12} on clearly defined cases, e.g. proven pernicious anaemia, post gastrectomy, malabsorption syndromes and dietary deficiency.[3,4,5] These cases were undoubtedly more clearly defined than many of the investigations currently undertaken which are due in large part to the introduction of electronic cell counters and with them accurate red cell parameters. The previously established specificity of the Euglena and Lactobacillus assays may now be regarded as overstated.[5]

Workers have concentrated on the vitamin B_{12} assays where several problems have been quite clearly identified e.g. Binder specificity for cobalamin, conditions of assays such as pH.[6,7] Relatively little attention has been given to the serum folate assay of radioisotope kits. Evidence has accumulated to indicate that the determination of the red cell folate is a more reliable and valid method of assessing the folate states of a patient than the serum estimation. Furthermore, results of the radioisotopic assay for red cell folate may be more clearly related to the folate states of a patient than that of the Lactobacillus assay[8]. This study emphasizes the high number of low serum folate levels found in hospital patients.

Methods

PATIENTS' SAMPLES

Ninety-six samples were analysed for vitamin B_{12} and serum folate. The samples were assayed on these patients on whom a brief clinical history, current medication, full blood count and blood smear examination could be obtained. These samples were firstly assayed by microbiological methods and were subsequently assayed by radioisotope kits in the majority of cases where the serum vitamin B_{12} level was less than 200 pmol/l, as this was thought to be the most relevant level to study. Serum folate values were obtained on all these samples.

Blood samples were collected into plain vacuum blood collection tubes. The serum was separated and stored deep frozen until assayed.

MICROBIOLOGICAL ASSAYS

The assay for vitamin B_{12} using *Euglena gracilis* is as investigated by Anderson.[2] Samples were assayed at 1/50 and 1/100 dilutions to aid in assessing serum inhibitors to the test organism. The assay for serum folate using *Lactobacillus casei* is that as investigated by Spray.[1]

RADIOISOTOPIC ASSAYS

The kits were:

(a) Diagnostic Products Corporation "Dual Count" using purified intrinsic factor as binder with a boiling step. β-Lactoglobulin is used as folate binder.

(b) Becton-Dickinson "Simultrac" using a binder containing R protein with the addition of blockers for the assay of true cobalamin. β-Lactoglobulin is used as folate binder. This kit also has a boiling step.

Table I
Vitamin B_{12} Assay — Normal Range Data

	Assay	Protocol
Euglena gracilis	103-660 pmol/l	
Simultrac (N=42)	140-738 pmol/l	162-694 pmol/l
	Deficiency?< 120 pmol/l	Deficiency< 125 pmol/l
Dual Count (N=42)	148-886 pmol/l	133-666 pmol/l
	Deficiency?< 120 pmol/l	Deficiency< 96 pmol/l

Results

For statistical analysis — results obtained from patients known to be on bactrim have been excluded. Normal range data from 42 normals is shown in relation to protocol values supplied by the manufacturers of the kits — Table I. The normal values represent a 95% confidence interval worked from percentiles. The serum folate values are shown in Table II. To assess assay precision 20 samples were assayed in duplicate at the normal range level and ten samples were assayed in duplicate in the subnormal range and coefficient of variation computed. This data is shown in Table III. The computed variances were tested by the F test to determine whether there was a significant difference betwen the assay precision. For vitamin B_{12} assays, at the normal level, the Euglena assay had the poorest precision (C.V. 14.2% $P<0.01$). At the subnormal level there was no significant difference between the three assays. The apparent difference in co-efficient of variation being due to the different mean values used in its calculation.

For serum folate, the Simultrac kit had the poorest precision at both levels ($P<0.001$).

Table IV indicates the number of subnormal vitamin B_{12} values obtained from the three assays with the number of patients having relevant clinical or haematological evidence for a low result. Excluding serum inhibitors the Euglena assay yielded the smallest number of subnormal values. Similar data for folate assays is summarised in Table V.

Table II
Serum Folate Assay — Normal Range Data

	Assay	Protocol
Lactobacillus casei	5.7-45 nmol/l	
Simultrac (N=42)	4.3-15.9 nmol/l	4.5-32.0 nmol/l
	Deficiency<4.3 nmol/l	Deficiency<4.5 nmol/l
Dual Count (N=42)	5.0-16.8 nmol/l	>6.8 nmol/l
	Deficiency<5.0 nmol/l	Deficiency<4.6 nmol/l

Table III
Vitamin B_{12} Assay — Precision

Assay	CV-%	Mean (N=20)	CV-%	Mean (N=10)
Euglena gracilis	14.2	254 pmol/l	32.2	90 pmol/l
Simultrac	8.1	234	21.8	127
Dual Count	7.1	233	14.1	158

Serum Folate Assay — Precision

Assay	CV-%	Mean (N=20)	CV-%	Mean (N=10)
Lactobacillus casei	7.5	6.9 nmol/l	10.0	4.1 nmol/l
Simultrac	15.0	7.0	28.3	3.8
Dual Count	8.5	8.0	7.6	4.7

Table IV
Vitamin B_{12} Assay — Low Values

Assay	No. of Low Values	Supportive Evidence
Euglena gracilis	12 (+ 5 I)	8
Simultrac	20	8
Dual count	17	9

Table V
Serum Folate Assay — Low Values

Assay	No. of Low Values	Supportive Evidence
Lactobacillus casei	21 (+ 3 I)	14
Simultrac	29	15
Dual count	21	14

Figure I summarises the vitamin B_{12} results. The solid line represents the lower limit of normal based on 95% percentile range. Figure II summarises the serum folate values. Figures III-VIII illustrate the correlation data along with batch means. Figures III and IV indicate that the Euglena assays yield lower vitamin B_{12} levels, with some samples exhibiting inhibition to the test organism not related to antibiotics. This was realised by the results obtained from the 1/50 and 1/100 serum dilutions. Figures VI and VIII illustrate that the *Lactobacillus casei* assay for serum folates produces higher values than do the radioisotopic assays.

Discussion

Measurement of vitamin B_{12} and folate is often part of an evaluation of patients with suspected neurological or mental abnormality, anaemia, malabsorption and suspected dietary insufficiency. In recent times reports have questioned the adequacy of commercial kits for routine measurement of serum cobalamin[7,9]. The kits employ a method which measures the extent to which combalamin in serum competes with radioactive isotope-labelled cyanocobalamin for binding sites on cobalamin-binding protein. The problems were outlined as being related to the nature of the cobalamin binding protein used and to the presence of biologically inactive cobalamin analogues as well as cobalamin in human serum.

Our experience with the two kits evaluated indicate that this problem as outlined appears to have been overcome by the use of purified intrinsic factor or the addition of blockers to the binder which contains the R-protein. More recently, other workers have suggested that many methods do not convert all serum cobalamins to cyanocobalamin and thus are not true competitive protein binding assays. No KCN in the extraction buffer results in a reduction in the vitamin B_{12} level in normal sera, whilst high concentrations of KCN (>30 mg/l) may cause a significant increase in the vitamin B_{12} level in serum from B_{12} deficient patients.[10]

Another error which has been reported for the assay of vitamin B_{12} using the raioisotope kits is that of high non-specific binding.[11] This problem is reported to be apparently due to incomplete inactivation of serum endogenous binding proteins and inadequate separation of free and bound radioisotope. This error means that falsely low serum B_{12} values will be obtained. This problem has been studied carefully during the trial undertaken in our laboratory and there is no reason to believe that this problem exists in either kit.

The correlation studies for vitamin B_{12} assays suggest that the introduction of purified intrinsic factor or blocking agents to R-proteins

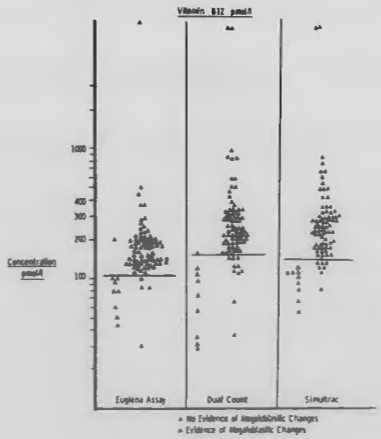
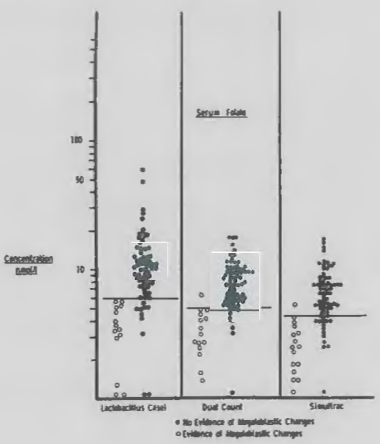


Figure I



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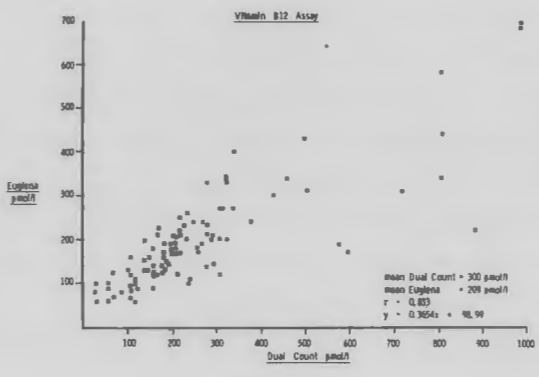

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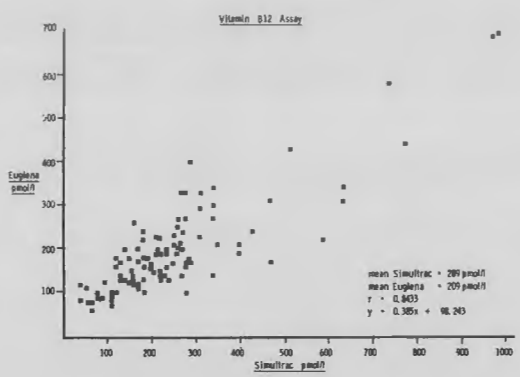

Figure IV

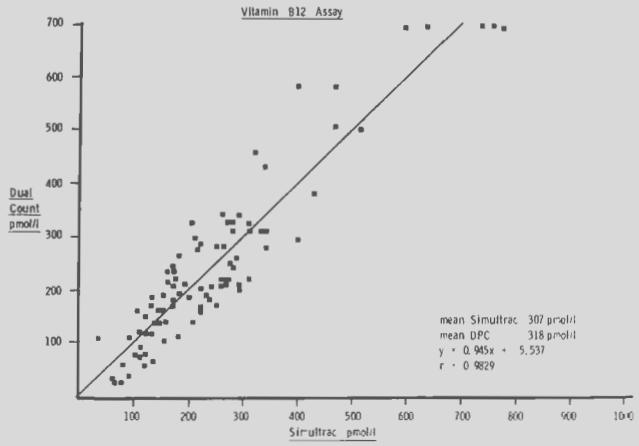

Figure V

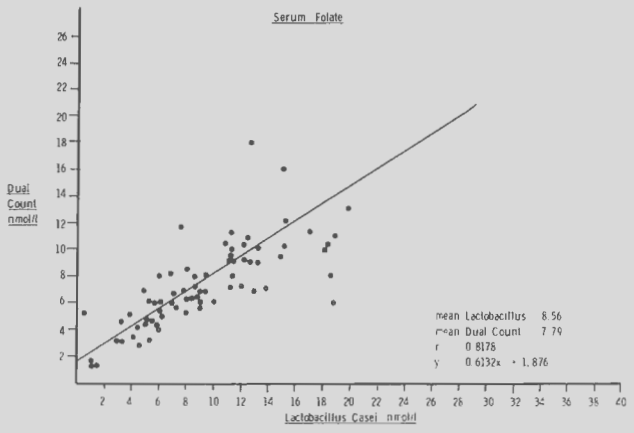

Figure VI

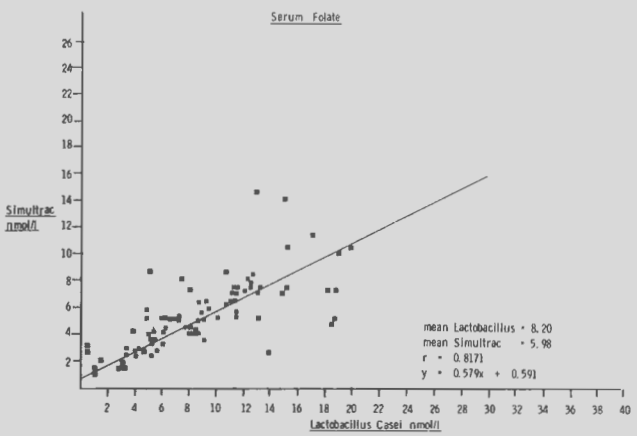

Figure VII

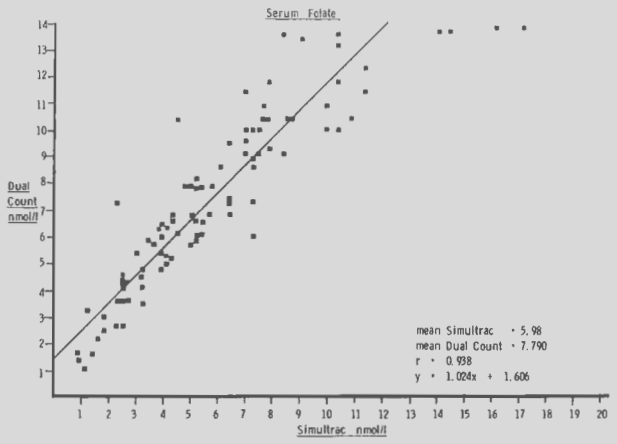

Figure VIII

as binders has not made the radioisotopic assays strictly comparable to the Euglena assay. In terms of clinical application the correlation is overall in agreement, although small differences still remain.

For the serum folate assay, the overall statistical correlation is adequate between the microbiological and radioisotope assays, although the Simultrac tends to place more patients in the 'deficient' category.

Any interpretation of a single serum B_{12} or folate evaluation must be approached with caution. There are documented conditions in which there may be a sub-normal serum cobalamin level when the patient is not cobalamin deficient e.g. 75% of vegetarians, 14-28% normal pregnant women[5]. Thus, a trial such as the one performed in our laboratory is by no means entirely adequate in assessing results from various assays with regard to the **true** cobalamin status of the patient.

In regard to the assessment of serum folate assays, serum folates were determined because our laboratory receives a far greater number of requests for serum folate rather than red cell folates even though the red cell folate more accurately reflects the folate status of the patient, particularly hospitalised persons[12].

The commercially available kits are suitable and appear to be reliable in detecting the cobalamin and folate deficient patients and are certainly advantageous for the testing of hospitalised patients where serum inhibitors in the form of broad spectrum antibiotics are a significant problem.

Acknowledgements

I would like to acknowledge the contributions of the staff of the Haematology Department, Middlemore Hospital in providing clinical and haematological data on patients referred for assays, and Dr I. Beer for his contribution in the assessment of results. Christine Curtis and Patricia Humphreys-Grey performed the microbiological assays.

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BORDETELLA PERTUSSIS: A Practical Methodology for Sampling, Isolation and Recovery of this Organism

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Introduction

This paper will review isolation rates from other countries, the situation in New Zealand and outline the most practical methods available for the isolation and recognition of *Bordetella pertussis* in the microbiology laboratory.

In England and Wales whooping cough became a notifiable disease in 1940. The institution of an immunisation programme resulted in a large decline of cases — from 35,000/year in the late fifties, when general use of the vaccine was instituted in the U.K., to a low of 2,000/year in 1976-77. In 1978-79 there was a sharp rise to 20,000 cases, followed by another dramatic decline in 1980. However, a notification in excess of 25,000 cases was recorded in 1981.[4] This concludes that "the recent epidemics in the U.K. have demonstrated that a decline in vaccination coverage in a previously highly vaccinated population can result in epidemics of pertussis".[4]

In the U.S.A. cumulative reporting of isolations of *Bordetella pertussis* indicated 1248 cases in 1981 and 1729 cases in 1982.[5] In the first six weeks of 1983, 111 cases have so far been reported — an increase of 25 cases over the same period for 1982.[6]

In New Zealand the isolation rate for *Bordetella pertussis* infections is not known, as there is no requirement to notify this disease, either as a clinical diagnosis or as a confirmed laboratory isolation.

In mid-1982 the local news media reported extensively that an "epidemic of whooping cough" was occurring in the Auckland area of New Zealand.

Symptoms:

The clinical manifestations range from persistent coughing climaxing in vomiting, severe choking, cyanosis and inability to breathe, to a mild, but persistent, cough.

The author's experience of isolations from specimens taken in New Zealand has indicated a wide age range — from babies of one month to several 40 year old adult patients. Most patients had bouts of coughing, usually culminating in a desire to vomit, but one of the 40 year old patients only exhibited a mild, chronic cough of some six months duration.

In the author's laboratory several isolates have come from infants under the age of three months — the recommended age for the commencement of immunisation.

Sampling:

(i) The classical method for the isolation of the organism is the cough plate technique. The author's experience of this technique has been that:
  (a) the medium is never available when required
  (b) inevitably the patient cannot/will not co-operate when the medium is available.

(ii) Throat swabs: not an ideal method, but practical experience has shown that the organism can be isolated from throat swabs if the technique of enrichment/isolation as outlined below is followed.

(iii) Pernasal/Nasopharyngeal swabs: now the preferred method[7,8], using the Medical Wire Ltd Pernasal swab MW160.

Nasopharyngeal Sampling:

The patient can be instructed to lie flat on a bed or couch. The swab is inserted slowly into the nasal passage maintaining a steady pressure. The operator will feel the swab 'pop' into the nasopharyngeal cavity, and the introduction of the swab into this correct area will invariably induce a coughing reflex in the patient.

In small children just over half the length of wire shaft will be in the nasal cavity and in adults well over two-thirds of the wire will be in the cavity.

Once the swab is in position it is preferable to leave it in place for 30 seconds to one minute, ignoring the patient's discomfort and pleas to remove it immediately.

Remove the swab and place into a non-selective transport medium prior to processing.

In some patients considerable resistance may be felt when the swab is in the area of the septum. Do not persist but remove and re-insert in the other nostril — the patient may have a deviated septum on the original side.

Culture Medium:

Bordet Gengou medium was described in 1905, modified in 1932 following the advent of penicillin, and has remained the first choice in the text books.[1] Regan & Lowe[7] processed 3237 specimens from August 1974 to April 1976 and obtained 1419 isolates of *Bordetella pertussis*. They noted during the survey that the Bordet Gengou medium available to them did not support the growth of test organisms recovered during the survey. All the isolates recovered came from a Charcoal Agar-Oxoid CM119-supplemented with added blood and cephalexin as the selective agent.

The success of the charcoal agar is probably due to the fact that the high concentration of the charcoal additive absorbs the toxic products of the bacterial growth. Regan & Lowe[7] re-emphasised the importance of the addition of 10% horse blood (whole cells) to the isolation/plating medium and 10% lysed horse blood to the transport/enrichment medium.

The overall advantage of this system was that whole blood produced larger sized colonies and the lysed blood produced smaller, but more numerous, colonies.

B. W. Lacey[2] employed a selective agent 4:4-diamidino-diphenylamine dihydrochloride at a concentration of 2μg/ml of medium. Penicillin 0.3 units/ml of medium has also been recommended by others in addition to the above compound.

Regan & Lowe[6] used cephalexin 40μg/ml of medium in their study and this agent was judged to be superior to penicillin alone, and methicillin alone in a paper published by Stauffer, Brown & Sandstrom.[6]

Charcoal agar should be prepared according to the manufacturer's instructions, ie 5.1g of Charcoal agar CM119 per 100ml of distilled water; bring to the boil to dissolve the agar; distribute in 20ml aliquots into universal screw capped containers. Sterilise and store on shelf until required.

When a plate is required melt the bottle of prepared agar base, allow to cool to 45°C, add 2.0ml of whole horse blood, add 1.0ml of cephalexin solution, mix thoroughly and pour into petri dish. Allow to set, dry the surface and inoculate when required.

Selective/Enrichment Medium:

Charcoal agar is prepared at half strength, ie 2.65g of Charcoal agar CM119 is dissolved in 100ml of distilled water and this is sterilised. The 100ml of sterile base is cooled to 45°C and then 5ml of cephalexin solution and 20ml of lysed horse blood is added. Mix thoroughly and distribute aseptically into sterile bijou bottles, label with the date and store in refrigerator. These bijous remain useful for 6 to 8 weeks from preparation.

Cephalexin Solution — 40μg/ml of medium:

Cephalexin is available as a reference powder through Lilly Industries (NZ) Ltd or can be purchased from the Sigma Chemical Company as Cephalexin monohydrate C4895 — the current (1983) price for 5g is $US10.75. To prepare the working solution 85.1mg of stock powder is dissolved in 100ml of sterile distilled water. 1ml aliquots are stored in the deep freeze in either small screw capped containers or autoanalyser cups plus lids. 1ml of the solution when added to 20ml of medium gives a final concentration of 40μg of cephalexin per ml of medium. The frozen solution will retain its potency for up to one year.

For laboratories that have a small demand for this medium, Mast Laboratories market a Selectatab-MS10. The medium can be prepared by sterilising 100ml of base, cooling to 45°C, and adding one Selectatab. Allow the tablet to dissolve, mix thoroughly and add the 10% volume of whole blood for the preparation of plates or lysed blood for the selective/enrichment stab. Each bottle of Selectatabs contains 25 tablets with a shelf life in excess of 12 months.

Laboratory Techniques:

In the author's laboratory the nasopharyngeal swab is received in a

non-selective transport medium. A charcoal agar plate is prepared as described previously and the swab is inoculated onto:

a. blood agar
b. chocolate agar
c. charcoal agar plate
d. inserted into the selective/enrichment agar stab.

a. & b. are incubated for up to 48 hours at 36°C in an atmosphere of added CO_2 and examined for routine respiratory pathogens — *Haemophilus influenzae* can cause an epiglottitis in all age groups.

c. & d. are incubated at 36°C for at least 72 hours and the plate is examined closely for any growth.

If there is no visible growth on the charcoal agar plate, or if there is growth but not characteristic of Bordetella, the swab from the selective/enrichment medium is reinoculated onto a fresh plate of charcoal agar prepared as above and then incubated for a minimum of a further 72 hours before a report that "no Bordetella isolated" can be issued.

Bordetella grows on the surface of the plate as a small pearly white colony after 72 hours incubation. A Gram stain will show the typical very small Gram negative bacillus and the oxidase reaction, using the paper strip technique with 0.1% tetra methyl-p-phenylenediamine dihydrochloride, is positive.

A suspension of the organism in normal saline is made on a slide and tested for agglutination with specific *Bordetella pertussis* antiserum — Burroughs Wellcome ZM10. When positive, the agglutination reaction is strong and rapid — about 10-15 seconds rocking will reveal the agglutination.

The author recommends using *Bordetella parapertussis* agglutinating serum as a negative control.

If necessary, the identification may be confirmed biochemically by inoculating MacConkey agar, nutrient agar, Simmon's Citrate agar, Christenson's urea agar and determining motility.

Bordetella pertussis is urease and citrate negative, non-motile, and does not grow on MacConkey agar or nutrient agar.

Bordetella parapertussis is urease and citrate positive, non-motile and does not grow on MacConkey agar.

Bordetella bronchiseptica is urease and citrate positive, motile and grows on MacConkey agar and nutrient agar.

Direct fluorescent antibody examination is not recommended as the sole diagnostic test as some 34% of positive cultures have been reported negative by F.A. on submitted smears[6,7].

Results:

From 1977 to 1981 the author collected and cultured 264 nasopharyngeal swabs from patients in the Dunedin geographical area. Four patients were positive in 1978, 10 patients in 1979, 16 patients in 1980 and 10 in 1981 — a recovery rate of 15.1%

From July 1982 to January 1983, 82 nasopharyngeal swabs have been collected in the Auckland area and 14 isolates have been confirmed — a recovery rate of 17%.

Discussion:

K. Fields[9] has reported a dramatic increase in isolation of the organism in Christchurch in 1981/82, and this has been confirmed in the outpatient population of the same city by D. Robertson[10].

It appears that *Bordetella pertussis* has shown a resurgence in New Zealand in common with the reported rates overseas. This resurgence was first noted in Dunedin, moved to Christchurch and has now appeared in the Auckland area.

It is recommended that microbiology laboratories reassess their ability to grow *Bordetella pertussis* and a proven, successful technique is fully described.

Acknowledgement:

The author acknowledges the technical support of the staff of the Microbiology Department, Medical Laboratory, Dunedin, and the Microbiology Department, Diagnostic Laboratory, Auckland.

Note:

Medical Wire Swabs MW160 and Mast Selectatabs are both available in New Zealand through the agent Carter Chemicals Ltd.

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Anion exchange microchromatography and Electrophoretic techniques for Haemoglobin-A_2 Estimation

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Abstract

This work was carried out in order to assess the reliability of the commercially available Helena Quik columns for the estimation of Haemoglobin A_2 (Hb-A_2). The electrophoretic-elution technique was the method used for comparison. Recently, a modification of methodology has been made in these kits with a noticeable change in Hb-A_2 values being obtained.

Normal samples were measured by the elution and kit methods along with eleven subjects with β-Thalassaemia minor. The unmodified kit (Kit A) yields Hb-A_2 levels which are consistently lower than those obtained by the electrophoretic elution method. This trend was not as obvious in the recently modified kit (Kit B).

The ability of the Helena Quik columns to detect the heterozygous β-Thalassaemia condition appears to be comparable to the electrophoretic method.

Key Words:

Thalassaemia minor, Haemoglobin-A_2, Microchromatography.

Introduction

The quantitation of Hb-A_2 is readily and reliably accomplished by electrophoresis of a haemolysate in a Tris, Edta, Glycine (Borate) buffer

at an alkaline pH (8.4-9.2) followed by elution of separated components, where relative concentrations are measured spectrophotometrically.[1,2]

The Helena Quik column kits utilising disposable plastic micro-columns for the estimation of Hb-A_2 have been commercially available in New Zealand for several years. In more recent months a minor modification to methodology has occurred in these kits which appears to have had a small but significant effect on the results obtained. The kit contains disposable plastic microcolumns filled with DEAE cellulose and appropriate buffer plus glycine-KCN-NaCl developer. Other workers have demonstrated that chromatographic techniques using either DEAE cellulose or Sephadex are suitable for an accurate and precise determination of the Hb-A_2 level.[3,2] The report by Moors et al (1979)[3] evaluates the commercially available Helena Quik columns for the measurements of Hb-A_2 along with the pasteur pipette method with starch block electrophoresis as the reference method. Generally, the electrophoresis followed by elution system gives consistent results but is dependent upon the use of a relatively fresh sample, consistency in cutting fractions and adequate time for elution of the haemoglobin.[1]

In general column chromatography is an accurate method which avoids the above problems but prior to the development of microchromatographic techniques had been too complex for the small routine laboratory.[4,5] The more recent short-column procedures using DE-52 anion exchange resin, Tris-HCl or glycine-NaCl-KCN developers are rapid, simplified procedures for determining Hb-A_2 levels.[6] They can be readily adapted to the use of commercially available stabilised haemoglobin controls with given values for the day-to-day monitoring of accuracy and precision.[7]

Materials and Methods

QUANTITATION OF Hb-A_2 FRACTION BY ELECTROPHORESIS AND ELUTION USING CELLULOSE ACETATE.

The method used in our laboratory is based on the procedure described by Marengo-Rowe (1965)[1] with minor modifications. The modifications are: (a) Use of Gelman micro-zone cellulose acetate strips. (b) Tris-Edta-glycine buffer pH 9.2-Gelman 'Haemoglobin Buffer'. (c) Elution of the Hb-A_2 fraction into 2 ml buffer.

This procedure requires a power pack capable of supplying direct current up to 10 milliamps at 200-300 volts, a tank suitable for electrophoresis of cellulose acetate strips 5.7 cm × 14.4 cm and a spectrophotometer with a narrow wavelength band at 413 nm.

The method used is as follows: 10 μl of freshly prepared haemoglobin solution at a concentration near 100 g/l is applied to the cellulose acetate strip which has been previously soaked in buffer. Using a constant current setting, a current of 2 milliamps per strip is applied for one hour to the cellulose acetate. Following the electrophoresis, the Hb-A_2 band is cut out and eluted into 2 ml of buffer and the major haemoglobin band is eluted into 20 ml buffer. The elution time used is at least one hour. The absorbances of the resulting haemoglobin solutions are determined in a spectrophotometer using cells of 1 cm path length at 413 nm.

MICROCHROMATOGRAPHIC DETERMINATION OF Hb-A_2 FRACTION (KIT A).

The method used is as described in the 'Helena Quik column' method sheet. The kit contains 50 disposable Hb-A_2 columns prepacked with DEAE cellulose in glycine buffer with KCN added. The kit also contains one bottle of developer A elution buffer containing glycine with KCN, one bottle of developer B containing glycine, NaCl and KCN, and one bottle of haemolysate reagent. The method may be summarised as follows: The resin is resuspended just prior to use. A 100 μl sample of haemolysate is applied to the resin surface, with care being taken to ensure that the resin is not disturbed. When the sample has been absorbed completely onto the resin, 1 ml of developer A is gently added to the column. Following the complete passage of developer A through the column, 1.5 ml of developer B is added to the column. The developer A elutes the Hb-A_2 fraction while developer B elutes the major haemoglobin fraction. The absorbance of each collection tube per sample is then determined in a spectrophorometer at 413 nm.

MICROCHROMATOGRAPHIC DETERMINATION OF Hb-A_2 USING KIT B.

The method used is as described in the 'Helena Quik column' method sheet. As in Kit A, this kit contains 50 disposable micro-columns pre-packed with DEAE cellulose in glycine buffer with KCN added. The kit contains one bottle of developer which is developer A in Kit A. A 100 μl sample of haemolysate is applied to the resin surface. When the sample is absorbed competely onto the resin, 2 ml of developer A is gently added to the column. The eluate, which contains the Hb-A_2 fraction is collected into a glass tube with a 3 ml volume mark. Following the sample application to the column, 100 μl of the same haemolysate is added to a glass tube with a 15 ml volume mark. This tube is then made up to the 15 ml mark with deionised water and the same is done for the 3 ml tube. The absorbance of the Hb-A_2 fraction (eluate) and the total haemoglobin is determined in a spectrophotmeter at 413 nm. a normal sample or a commercially available control with a known value was assayed with each batch.

Calculations

Kit A.

$$\% \text{ Hb-}A_2 = \frac{\text{Abs. Hb-}A_2 \text{ fraction}}{(\text{Abs. total Hb} \times 5) + \text{Abs. Hb-}A_2 \text{ fraction}} \times 100$$

Kit B.

$$\% \text{ Hb-}A_2 = \frac{\text{Abs. Hb-}A_2 \text{ fraction}}{\text{Abs. total Hb} \times 5} \times \frac{100}{1}$$

where in each kit, 5 is the dilution factor, i.e. 15 ml total fraction tube and 3 ml for Hb-A_2 fraction.

Sample Selection

The normals were obtained from the routine Haematology laboratory where the blood samples had been processed for full blood counts. Samples were chosen in which the haemoglobin mean cell volume and mean cell haemoglobin were all within the normal range.

The β-Thalassaemia samples had been referred to our laboratory for a "Thalassaemia screen". The data presented comes from those samples where the haemoglobin level and mean cell volume were below the normal range and a raised Hb-A_2 level was obtained on multiple testing using our routine elution method with appropriate controls. In each case no Haemoglobin-H bodies had been found on examination of a suitably stained smear. The Thalassaemia samples measured in Kit B are from different patients with β-Thalassaemia minor than those measured in Kit A. Thus, a direct comparison for correlation of the two sets of results was not possible. The samples measured using Kit B were, however, tested in parallel by the elution method.

Table I
Normal and β-Thalassaemia Ranges

Normal	Kit A Micro-Column	Kit B Micro-Column	Elution
No. Samples	27	27	18
Range	1.1-2.7%	1.4-2.8%	1.9-3.4%
Mean±2SD	1.2-2.7%	1.6-2.9%	1.9-3.6%
Mean	2.0%	2.3%	2.8%

β-Thalassaemia Minor	Kit A	Elution	Kit B	Elution
No. Samples	11	11	11	11
Range	2.9-4.5%	3.9-6.3%	3.7-5.8%	4.1-6.6%
Mean±SEM	3.7%±0.124	4.2%±0.25	4.8±0.185	5.4±0.231

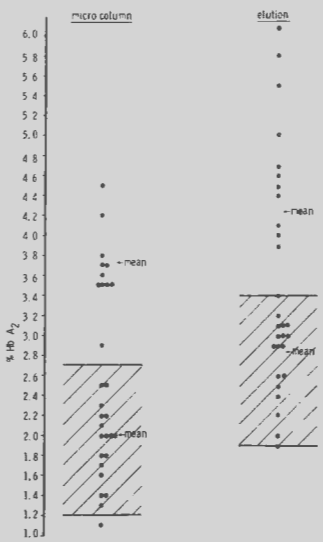

Figure I

Table II
β-Thalassaemia trait — Hb-A_2%

Kit B Micro-Column (N=11)	Elution (N=11)
4.3	6.2
5.2	4.9
5.2	6.6
4.1	5.4
5.4	4.8
4.7	5.1
4.8	5.3
4.8	5.8
5.8	6.4
5.2	4.9
3.7	4.1

Results

Table I summarises the data from the normal and the β-Thalassaemia minor groups. In the normal group, Kit A and the elution method were run in parallel on the same samples. The data from Kit B comes from a different set of normals. In the β-Thalassaemia group, in each case the micro-column and elution methods were run in parallel. However, the samples for Kit A and Kit B are from a different set of patients with β-Thalassaemia minor. The mean ± standards error is used in the β-Thalassaemia group since the number of samples was considered to be too small to use the standard deviation. The data demonstrates that Kit B generally gives slightly higher values than Kit A in the normal group. However, the difference is small. Figure I presents the results from 29 samples tested in parallel using Kit A and the elution technique. The shaded portion represents the normal range determined for both techniques. The data from Kit B is excluded from this Figure since the two micro-columns were not run in parallel on the same samples. The data illustrates the tendency for the microchromatographic technique (Kit A) to yield lower Hb-A_2 levels in both the normal and β-Thalassaemia carriers than the elution method. Table II illustrates the same trend in the β-Thalassaemia group of 11 patients where Kit B and the elution method were tested in parallel. The individual Hb-A_2 levels are given. The same data is seen in summary form in Table I.

The correlation data for Kit A and the elution method is presented in Figure II. There is a strong correlation between the two methods with an r value of 0.9128. The regression line is represented by the equation $y=0.7547+1.0863x$.

The Canterbury Scientific Co. have made available Hb-A_2 controls which have been stablisied with carbon monoxide. Values for each control are given with some batch to batch variation as 1.8%, 3.8%, 4.5%. The manufacturers state that the samples may variably equilibrate with oxygen during column chromatography to give aberrent results. Eluates should therefore be bubbled with carbon monoxide prior to measuring the absorbances. These controls have been measured, without bubbling with carbon monoxide prior to measuring absorbance, by the two kits and the elution method to determine the extent of this potential problem. In an attempt to assess this, precision studies on a batch-batch basis were carried out. The results are summarised in table III. A haemolysate prepared from a normal sample was also assayed so that a comparison could be made with the stabilised Hb-A_2 controls. Kit B has been studied in parallel with the elution method. Except for the 4.5% Hb-A_2 control, insufficient data from Kit A was obtained for any valid conclusions to be reached. At the 4.5% control level for Kit A, a

Table III
Precision studies

	Kit B	Elution
1.8% Control: Range	1.2-2.6% (N=10)	1.1-2.5% (N=12)
Mean	1.8%	1.8%
Co-efficient of Variation	21.0%	20.5%
3.8% Control: Range	2.7-5.1% (N=13)	3.2-4.2% (N=12)
Mean	3.7%	3.9%
Co-efficient of Variation	17.6%	8.4%
4.5% Control: Range	3.8-5.6% (N=13)	4.2-5.5% (N=18)
Mean	4.5%	4.8%
Co-efficient of Variation	9.7%	9.2%
Normal Sample: Range	1.8-2.9% (N=12)	2.3-2.9% (N=12)
Mean	2.5%	2.6%
Co-efficient of Variation	13.5%	11.0%

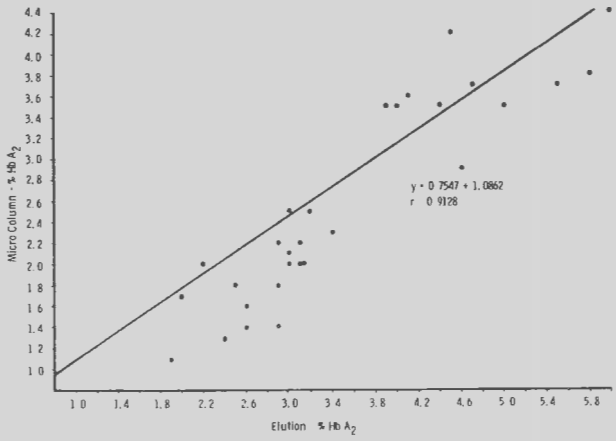

Figure II

mean result of 3.8% was obtained with a co-efficient of variation of 16.2%. This appears to be considerably different from the other two results and may well be linked to the re-equilibration problem. However, the results obtained from Kit B and elution appear to be comparable, both in terms of mean values and co-efficient of variation, suggesting that re-equilibration with oxygen is not a significant problem with the revised protocol.

It is of interest to note that at the 3.8% level, the micro-column method has a much larger co-efficient of variation at 17.6% than the elution method.

Discussion

A review of previously published data from other workers using anion exchange chromatography indicates that slightly higher Hb-A_2 levels have been obtained in samples from patients with β-Thalassaemia minor than those from our laboratory. However, the more recently modified kit (Kit B) yielded slightly higher Hb-A_2 values than the previous kit (Kit A) which has led to a closer agreement with the reviewed data.

In one report using anion exchange chromatography, involving over 1300 normal adults and more than 50 previously diagnosed β-Thalassaemia, the normal range was determined as 1.7-3.5% Hb-A_2 with a mean of 2.6%. Those with β-Thalassaemia minor gave a range of 3.9-6.5% Hb-A_2 with a mean of 5.1%.[2] Our results using the Helena Quik columns are in relative agreement with these figures but tend to be a little lower using Kit A, a value of 2.9% as the upper limit of normal was obtained as opposed to 3.5%. Similar Hb-A_2 levels have been reported using micro-columns of Efremov et al (1974)[7]. They reported that in the β-Thalassaemia minor group a Hb-A_2 range of 3.5-8.3% was obtained. Haemoglobin A_2 levels using anion exchange chromatography have also been reviewed by Efremov — Efremov (1974)[8], where similar values are quoted.

The work reported by Marengo-Rowe (1965)[1] employing cellulose acetate electrophoresis followed by elution of the Hb-A_2 fraction gives a normal range of 1.0-2.0% and a range of 3.5-7.0% for patients with β-Thalassaemia minor. A further report by Alperin et al (1977)[9] employing the same elution technique, gives a normal range of 1.9-3.5% for Hb-A_2. On a group of 111 known β-Thalassaemia minors a range of 3.5-6.9% Hb-A_2 was determined. The data from our laboratory is in close agreement with these figures.

An extensive report by Moors et al (1979)[3], evaluates microchromatography using pasteur pipettes and the commercially available Helena Quik columns with starch-block electrophoresis as the reference method. They found that the mean Hb-A_2 levels obtained by the two methods did not differ significantly although the standard deviations were in general larger for microchromatography than for starch-block electrophoresis. Furthermore, they demonstrated that for the commercial micro-columns an over-estimation of 18% in relation to their reference method occurred over the whole range of physiological Hb-A_2 levels. This has not been our experience with either of the kits and so may well be related to differences in the two electrophoretic methods used to determine the Hb-A_2 level. It is possible that the elution method using cellulose acetate results in a slight over-estimation of the Hb-A_2. In the same report, duplicates for Hb-A_2 levels on parallel testing were shown to be closer for the commercial columns than with the pasteur pipette method and comparable with the determinations made in starch-block, for this series.

The run-to-run precision study carried out in our laboratory indicated that at a mean value of 2.6% the co-efficient of variation is smaller for

the elution method, but at the 4.5% level the co-efficient of variation for the micro-column method is the same as for the elution method. Thus, both from published data and our own studies, it would appear that microchromatographic and electrophoretic techniques are satisfactory methods for determining Hb-A_2 and that correlation between the methods is good. The commercially available kit method represents a further refinement of the short-column procedure and significantly shortens the time required for Hb-A_2 measurement, and is more than satisfactory in detecting the patient with β-Thalassaemia minor.

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The Air Handling Products Department of Email Limited introduces a new range of Aipure clean benches. The range includes horizontal and vertical laminar flow units, Class I biohazard cabinets, Class II systems for the protection of both product and operator and a clean bench designed specifically for handling Cytotoxic drugs.*

For further information on the Airpure clean bench range contact the Air Handling Products department of Email Industries, 6 Monahan Road, Mt Wellington, Auckland, phone 574-037, or send the coupon.

*All cabinets conform to the required standards with a certified service for the ongoing testing of the units to ensure both filter and unit integrity.

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LABORATORY SAFETY

Recently, Mike Collins from the Public Health Laboratory at Dulwich, London, gave a series of lectures throughout New Zealand on Laboratory Safety.

For those who were unable to attend these lectures, reproduced below is some of the relevant material which was given.

BARRIERS AGAINST LABORATORY-ACQUIRED INFECTIONS AND THE ESCAPE OF ORGANISMS INTO THE COMMUNITY

PRIMARY BARRIERS	Around the organisms. Good Laboratory Practice (GLP). Safety cabinets
SECONDARY BARRIERS	Around the worker. Protective clothing. Good hygiene. Immunisation
TERTIARY BARRIERS	Around the laboratory. Security. Good design. Good ventilation. Vermin proofing. Safe waste disposal

ROUTES OF INFECTION

INGESTION	Splash to bench to hand to mouth Pipette to mouth Pipette to fingers to mouth
INOCULATION	Syringe and needle Broken infected glassware Cuts, abrasions, very small lesions on skin
INHALATION	Aerosols, infected air-borne particles

PROCEDURES AND ACCIDENTS WHICH RELEASE INFECTIOUS AGENTS INTO ENVIRONMENT

These may result in contamination of hands, bench, equipment and the release of aerosols.

Work with loops, making slides, agglutination tests, plating.
Pipetting, blowing out the last drop.
Use of syringes, leaking barrel, discharge of contents, ejection of needle.
Centrifuging, tubes too full, breakage.
Opening screw capped bottles and petri dish cultures.
Using poorly maintained homogenisers.
Pouring liquids, even into disinfectants.
Leaking specimens and cultures.
Breakages of any kind.
Inoculating animals.
Autopsies on animals.

PERSONAL PROTECTION AND LABORATORY CLOTHING

The sensible laboratory worker will seek to protect his person and his clothing from the chemicals, pathological and infectious materials which he handles. The good employer will provide the proper facilities and the good manager will ensure that these facilities are correctly used.

Prejudices may have to be overcome, the ignorant may have to be enlightened and those who put fashion and appearance before safety must be made to see the errors of their ways. Management must be persuaded to provide enough hand basins and other washing and sanitary facilities to meet the elementary requirements of laboratory hygiene.

The problems of accommodating out-door clothing, shopping bags and other things people bring into laboratories may be solved by the provision of lockers and cupboards placed intelligently, and this necessitates good laboratory planning and sensible management.

The traditional white coat offers limited protection, not only in its design but in the way it is worn and altered to suit current fashions. The tapes that tie gowns at the back may become knotted and zip fasteners may jam, offering a serious hazard if it becomes necessary to remove the gown or coat in a hurry, e.g. in case of fire or chemical spillage.

The basic requirements of a good, safe laboratory coat are:—

1. Easily removable
2. Covers neck and chest
3. Does not gape at the knees when the wearer is sitting
4. Fits tight at the wrist to avoid aerosol infection of arms
5. As fire and chemical resistant as possible

CARCINOGENIC AND TOXIC HAZARDS OF CHEMICALS

EXPOSURE

Can occur from:— inhalation of dust or vapour
absorption through the skin
ingestion from contaminated hands
absorption from contaminated clothing
contact with contaminated benches, floors and apparatus

CHEMICAL AND PHYSICAL FACTORS AFFECTING ABSORPTION
Purity/concentration of active substances
Synergistic effect of vehicle/solvent
Effect(s) of impurities
Solubility/particle size
Sensitizing potential
General properties eg density, volatility

ROUTES OF ABSORPTION
Percutaneous
Pulmonary
Oral

FACTORS AFFECTING PERCUTANEOUS ABSORPTION
Area of exposure/coverage
Integrity and condition of skin
Hydration and pre-treatment
History of allergy
Properties of the chemicals

RISK OF DEVELOPING A TUMOUR
In general, proportional to:—
Length and frequency of exposure
Concentration of the chemical

CHEMICALS WHICH MAY CAUSE TUMOURS OF THE URINARY TRACT

α — naphthylamine	β — naphthylamine
benzidine	4 — aminodiphenyl
4 — nitrodiphenyl	o — dianisidine
o — tolidine or their salts	

nitrosamines, nitrosophenols, nitronaphthalenes

CATEGORIES IN RELATION TO RELATIVE ACUTE TOXICITIES

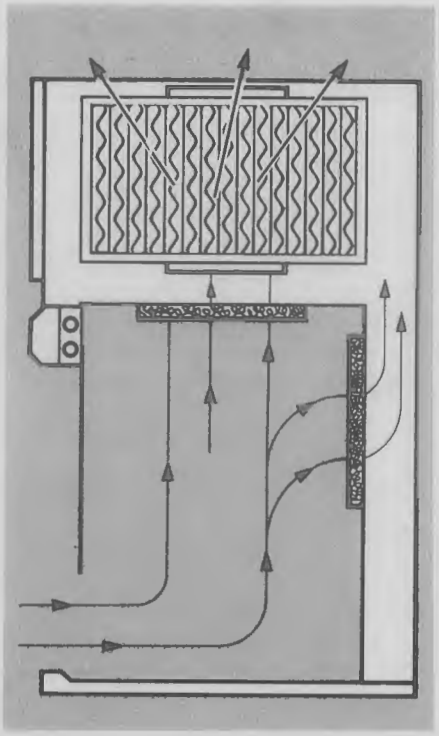
Toxicity rating	Term of toxicity	Probable human lethal dose for a 70 kg man	Compound belonging to the group with oral LD50 for rats in mg/kg	
1	practically non-toxic	>15 g/kg	propylene glycol.	26,000
2	slightly toxic	5-15 g/kg	sorbic acid:	7,400
3	moderately toxic	0.5-5.0 g/kg	isopropanol:	5,800
4	very toxic	50-500 mg/kg	hydroquinone:	320
5	extremely toxic	5-50 mg/kg	lead arsenate:	100
6	supertoxic	<5 mg/kg	nicotine:	50

PROTECTION FROM TOXIC HAZARDS
Sound education and training
Good hygiene and housekeeping
Use of protective clothing
Use of safety devices
(Monitoring of environment)
(Analysis of biospecimens)

Gelman

BIOGARD Class I Cabinet
BSC Series MK II

manufactured in compliance with AS 2252, Part 1 – 1981



Applications
The BIOGARD MARK II Biological Safety Cabinet is designed to provide a high degree of operator protection against risks posed by the handling of hazardous microbiological agents defined as ordinary and special hazards.

Operation
Personnel protection is provided by an inward flow of air through the work access opening thereby creating an effective barrier between the operator and the potentially contaminated work zone. Air from the work area is then directed through prefilters and a HEPA filter before being discharged into the laboratory.

Construction
- Gas tight cabinet construction — continuously welded. Finished in double baked enamel.
- Work zone manufactured in Series 300 stainless steel with 2B finish.

Physical Data

Model		Height mm	Width mm	Depth mm	Weight kg
BSC-99	Overall	1150	1165	700	180
	Work zone	700	940	600	
BSC-191	Overall	1300	2105	600	275
	Work zone	700	1880	600	

- Cabinet exhaust fitted with HEPA filter to remove 99.99% of all particles 0.3 micron and larger.
- Audible alarm incorporated to signify loss of safe air flow conditions.
- Front sealing panel supplied with unit to close work area when not in use.
- Magnehelic gauge fitted to indicate condition of prefilters and exhaust HEPA filter.
- Variable speed high performance motor/blower with solid state control.

Special Features
- Compact dimensions to enable access through standard door openings.
- Two prefilters to extend HEPA filter life and enhance uniformity of air flow through the work zone.
- One piece stainless steel work surface with non-spill front edge.
- Exhaust HEPA filter located at side of cabinet for ease of service access.
- Vertical viewing window to eliminate visual distortion through reflection.
- Two models for single or dual operator applications.

GIBCO™ NEW ZEALAND LIMITED
18-24 Botha Road, P.O. Box 12-502 Penrose, Auckland 6, New Zealand
Telephone 593-024 Auckland Telex: NZ 21343

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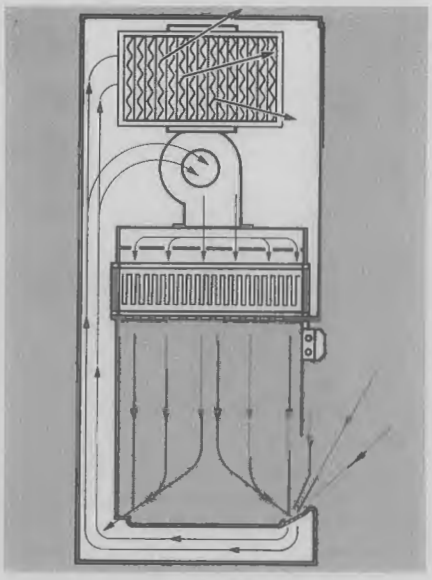
BIOHAZARD Class II Cabinet
BH Series MK III
manufactured in compliance with AS 2252, Part 2 – 1980



Applications
The BIOHAZARD BH SERIES are Laminar Flow Biological Safety Cabinets designed to provide a high degree of protection for personnel, products or experiments and the environment from risks associated with hazardous microbiological agents which may be defined as ordinary and special hazards.

Operation
Sterile and particle free air is recirculated in the work zone in a vertical Laminar Flow manner providing product protection. An air barrier is created between the operator and the work zone by an in-flow of room air through a purpose designed grille which spans the full width of the access opening thus providing operator and environment protection.

Exhaust air from the cabinet is HEPA filtered before being returned to the laboratory.

Construction
- Continuously welded gas tight cabinet finished in double baked enamel.
- Work zone manufactured in Series 300 stainless steel with 2B finish. Removable work floor with liquid retaining lip.
- Two variable speed motor/blowers fitted to provide individual adjustment of airflow through laminar flow and exhaust HEPA filters. Both filters provide a minimum efficiency of 99.99% on particles 0.3 micron and larger.
- Audible alarm to signify failure of safe airflow conditions.

Physical Data

Model		Height mm	Width mm	Depth mm	Weight kg
BH143	Overall	1490	1440	765	300
	Work zone	610	1180	575	
BH204	Overall	1490	2050	765	420
	Work zone	610	1790	575	

- Front sealing panel supplied with unit to close work area when not in use.
- Magnehelic gauge fitted to indicate pressure drop of main and exhaust HEPA filters. Two way selector valve enables a check on the condition of either filter.
- All zones under positive pressure are surrounded by negative pressure zones to maximise containment.

Special Features
- Compact overall dimensions and smooth cabinet profile enables easy access through standard doorways and where ceiling height is limited.
- Removable one piece non-perforated work floor for ease of cleaning. Stable, vibration-free work surface.
- Exhaust HEPA filter located within main cabinet housing and easily accessible for servicing.
- Aerodynamically designed air barrier grille for maximum air barrier effectiveness.
- Selector valve for magnehelic gauge located to enable ready access from normal operator position — obviating need to lean into cabinet to check filter condition.
- The total work area is manufactured in stainless steel and is free from crevices thereby eliminating possible accumulation of biological matter.

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DISINFECTANTS IN MEDICAL LABORATORIES

A. Chemical disinfectants should NOT be used:—

1) When sterilization is required
2) When physical methods can be used instead
3) When thorough cleaning is adequate
(Note:— Higher level of contamination in laboratories)
4) When disposable equipment can be economically used

B. Indications for use of disinfectants

1) Skin
Venepuncture — swabs and sprays
Lesions (in P.M. room) — after initial promotion of bleeding
Hand washing areas — Bar of soap **or** soap dispensers

2) Instruments and apparatus
Centrifuge bowls
Cryostats
Cytocentrifuges
Shakers
Automated equipment
E.M. grids and Holders
Equipment for servicing

3) Infected items prior to handling
Discard jars for pipettes (large and small)
for disposable loops
Sampling cups for automated apparatus
Dialysis membranes
Autoclave drains

4) Decontamination of Surfaces

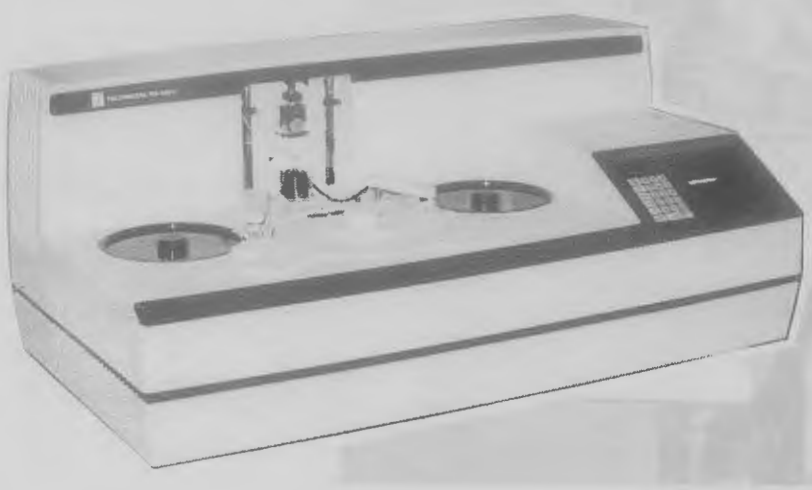
General Hygiene
Specimen reception areas
Specimen trays — internal and external
Covers for shakers
Contaminated areas
Spillage of specimens or cultures
24 hour urine bottles
Sinks and slop hoppers
Histology cutting boards
Histological tissue
P.M. tables, scales
P.M. clothing, boots, aprons, heavy duty gloves
Increased risk areas
Microbiological protection cabinets
P.M. rooms, gulleys, drains, equipment, benches
(after a Typhoid case)
Serum hepatitis containing material
Cadavers from category A infected cases

C. Activity of Chemical Disinfectants

Disinfectant Group	Activity Against:- Gram + Bacteria	Gram − Bacteria	Acid Fast Bacteria	Spores	Viruses	Activity in Presence of Protein	Uses	Limitations
Alcohols	++	++	++	−	+	++	Skin	
Aldehydes								
1) Formaldehyde	++	++	++	+	++	++	Many	Toxic to inhale
2) Gluteraldehyde	++	++	+	++	++	++	Viruses	Expensive
Ampholytes	++	+	0	0	0	0		
Chlorexidine	++	+	+/−	0	0	0	Skin	Easily inactivated
Halogens								
1) Hypochlorites	++	++	++	++	++	+/−	Viruses	Corrosive Easily inactivated
2) Fichlor Clearon	++	++	++	++	++	+/−	Spillages	Wet spills
3) Iodine Compounds	++	++	−+	+	++	+/−	Skin	Easily inactivated
Phenolics								
1) Chloroxylenol	+ ±	±	0	0	0	−/		
2) Clear, Soluble	−+	+ -	+	-/	-	- +	general	Not all viruses
3) White Fluids	+	++	+	+/−	+	++		Toxic- 'smelly'
Pine Fluids	±/−	+/−	0	0	0	0		Inactivated
Quaternary Ammonium	+ +	+	0	0	0	0		Inactivated
Q.A.C's + Diguanides	+	-	0	0	0	0		Inactivated

D	Uses	Concentration
Alcohol	Skin	70%
Chlorhexidine	Skin	0.5% in 70% spirit
Povidone-iodine	Skin	as supplied
Formaldehyde	*Solution* Tissue	4% (10% Formalin)
	Vapour Protection Cabinets	20% (50% Formalin)
Gluteraldehyde	Metal apparatus and areas contaminated with Blood & serum	2% by the addition of buffer pH 7.0-8.0
Hypochlorites	Bench areas contaminated with a little blood and serum	1,000 p.p.m. (1%)
	Contaminated areas, sinks spillage of blood	10,000 p.p.m. (10%)
Clear Phenolics	General areas	1-2%
	Organic material Tuberculosis and heavily contaminated areas	2-5%

RANDOM ACCESS



The TECHNICON RA1000 is now in use in Australia and New Zealand and the list of users is growing rapidly.

The TECHNICON RA1000 selectively and quickly analyses serum and urine samples for a wide range of biochemistry tests without batching samples.

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SAFETY AUDIT

Part 1. General and Chemical

1. *Fire precautions*

Are fire alarms fitted?
Are all exits maintained to provide free and unobstructed egress from all parts of building?
Are all exits free of locks or fastening devices that could prevent free escape?
Are fire doors kept closed?
Do fire doors conform to fire regulations?
Do all exits open directly to a street, yard, court, or other open space?
Are all exits marked by proper signs and illuminated?
Is access to exits marked in all cases where the exit or the way to reach it is not immediately visible?
Are exit signs obscured e.g. by equipment or furniture?
Is exit access arranged so that it is not necessary to travel toward any high hazard areas to escape?
Are corridors and passages maintained clear and unobstructed for movement of personnel and fire fighting equipment?
Are walls, floors and ceilings constructed to withstand a one hour fire?
Will doors withstand a half-hour fire?
Are portable fire extinguishers maintained fully charged and operable and kept in designated places at all times?
Are fire extinguishers conspicuously located, readily accessible, and available along normal paths of travel?
Are extinguishers and locations conspicuously marked to indicate intended usage?
Are all extinguishers placed so that the instructions face outward?
Are extinguishers available suited to the class of fire anticipated in each area?
Are extinguishers placed according to distances for proper coverage?
Are extinguishers inspected, maintained, and replaced by spares on the same day that they are discharged?
Are laboratory rooms with potential fire hazards equipped with proper extinguishers for emergency situations?
Are hose outlets within easy reach of a person?
If flammable liquids are used in a laboratory, is the mechanical ventilation sufficient to remove vapours before they reach a hazardous concentration?
Is there a designated fire officer for each department or building?
When was the last fire drill?
When were staff last instructed in use of fire-fighting equipment?
Are 'fire notices' and instructions prominently displayed?
Are No Smoking signs posted in prohibited areas?
How often is there a routine fire inspection by a professional fire officer?
Are employees trained to leave premises obscured by smoke?
Do they know the hazards of high concentrations of CO_2 after using CO_2 extinguisher?

2. *Working Environment*

Are all places kept clean and orderly and in a sanitary condition?
Is every enclosed work place so constructed and maintained to prevent entrance and harbourage of rodents, insects, and vermin?
Are toilet facilities adequate for both sexes and in accordance with building regulations?
Are all floors kept clean and dry?
Are all floors, areas, and passageways free from protruding nails, splinters, holes, and loose boards?
Are aisles and passageways clear of all obstructions and in good repair?
Are aisles and passageways wide enough to operate equipment safely?
Do fixed stairs make an angle to the horizontal of between 30° to 50°?
Are flights of stairs having 4 or more risers equipped with railings or handrails?
Are stair treads reasonably uniform and slip-resistant?
Are all exposed steam and hot water pipes covered with an insulating material, or guarded?
What insulating material is used on pipes?
Is there a handbasin in each laboratory room?
Are changing rooms provided for each sex where necessary?
Does each employee have a lockable clothing cupboard?
Are pegs provided for laboratory protective clothing or is this clothing kept in staff lockers.
Is a staff room provided and adequately furnished with facilities for eating and drinking?
Are cleaners allowed into rooms containing toxic or infectious hazards?
Are cleaners on the laboratory or other (e.g. outside contractors') staff?
Are safety showers (drenches) provided? Are they accessible?

3. *Personal Protection*

Are sufficient overalls or other protective clothing provided?
Is it well designed?
Are gloves of suitable pattern provided for work normally or exceptionally requiring hand protection?
What are the first aid facilities — immediate and local, or distant?
Are first aid cabinets adequately stocked?
Is there a drill for a medical emergency or for obtaining help if there is a serious accident? Do people know who to telephone?

4. *Eye Protection*

Are eye wash fountains or hoses provided? Are they in sensible places? Do the staff know where they are?
Have staff been instructed in their use, removal of contact lenses, time of wash, need to roll the eyes?
Are goggles or face shields provided for all occupants of each room where chemicals are handled?
Are there rules about wearing eye protection?
Are goggles provided for visitors?

5. *Electrical Hazards*

Is all electrical equipment installed and maintained by a qualified electrician?
Is there evidence of do-it-yourself electrical work?
Are all new electrical installations and all replacements, modifications, or repairs made and being maintained in accordance with the local Electricity Board requirements?
Does the interior wiring system have a grounded conductor? i.e.: 3-wire system?
Do all electrical applicances comply with accepted standards?
Are the cords of all electrical equipment in good condition, not frayed or spliced, etc.?
Are cords used properly (not run under rugs or under heavy equipment)?
Is there only one plug per socket outlet, i.e.: no multiple adaptors to sockets?
Are the lighting levels such that good illumination is provided in all walking, working, and service areas to ensure safety?
Are circuit breaker panels and cut-off switches located so as to be readily accessible?
Are all circuit breaker switches marked or labelled?

6. *Flammable Liquids*

Are flammable liquid containers constructed of noncombustible materials?
Are storage drums vented?
Are flammable liquids stored in proper containers?
Are flammable liquids stored on benches overnight?
Are flammable liquids stored on shelves over benches?
Are storage cabinets being used for storing flammable liquids in laboratory rooms?
Are storage cabinets labelled "FLAMMABLE — KEEP FIRE AWAY"?
Is the storage area provided with either a gravity or mechanical exhaust ventilation system?
Are extinguishers available where flammable or combustible liquids are stored?
Are "NO SMOKING" signs posted in the flammable or combustible liquid storage areas?
Are all refrigerators in labs marked modified for the purpose of flammable liquid storage?
Is care taken not to store any flammable liquids in unmodified refrigerators?
What are disposal arrangements for used solvents (e.g. sink, contractor, burned)?
Is legislation about highly flammable liquids known to staff?

7. *Storage*

Is storage of material such that it is stable and secure against sliding, collapse, falls, or spills?
Are storage areas kept free from accumulation of materials that

constitute hazards from tripping, fire, explosion, or pest harbourage?

8. *Flammable, Compressed and Liquefied Gases*
   Are hydrogen cylinders secured to prevent movement?
   Is the hydrogen storage area permanently marked "HYDROGEN — FLAMMABLE GAS — NO SMOKING OR OPEN FLAMES"?
   Is the hydrogen system in an adequately ventilated area?
   Is each portable gas container legibly marked with the name of contents — example: Hydrogen?
   Are compressed gas cylinders determined in safe condition by visual and other inspection required in regulations?
   Does each compressed gas cylinder or tank have an installed pressure relief device?
   Are all compressed gas cylinders stored and secured so they cannot fall?
   Are protection caps in place on compressed gas cylinders except when in use?
   Are compressed gas cylinders always moved on hand trucks, carts, dollies, etc.? (They must never be rolled or dragged.)
   If more than one cylinder of a flammable gas (hydrogen, acetylene, etc.) is used in a laboratory room, has permission been granted by the Safety Officer?
   Are laboratories that use toxic gases equipped with proper gas masks and respirators?
   Are all empty gas cylinders marked "EMPTY" or "MT"?
   Is there more than one LPG container per room?
   Are LPG containers in safe areas?
   Are staff aware of the hazards of oxygen-rich and nitrogen-rich atmospheres?
   Are liquid O_2 and N_2 stored and used in well ventilated rooms?
   Do staff wear protective clothing and gloves when handling liquified gases?
   Do they know the first aid for cryogenic burns?

9. *Toxic Carcinogenic and Hazardous Chemicals*
   Is food and drink prohibited in the work area?
   Are known toxic and carcinogenic chemicals so labelled?
   Are they properly handled and stored?
   Are gloves provided?
   Is the protective clothing adequate and safely disposed of?
   Do the staff know which chemicals cause intoxication, vertigo, stupor, neurological disorders?
   Are cyanide antidotes provided?
   Are perchloric acid, perchlorates and other chemicals subject to the production of shock-sensitive compounds, such as ethers properly stored?
   Are chemicals with a limited shelf life (e.g. 90 days for ether) properly dated and disposed of?
   Are ovens kept separate from flammable liquids?
   Do staff know how to clean up spilled flammable liquids?

10. *Radiation Protection*
    Is there a Radiation Protection Officer?
    Is each employee who works in an area where radioactive material is used furnished and wearing film badge?
    Are skin and clothing of employees monitored?
    Is radiation exposure of individuals to the body limited to 1 1/4 rems per calendar quarter?
    Is each radiation area posted with the proper radiation caution sign?
    Are all radiation area employees instructed in the safety problem, precautions, and devices to minimize exposure?
    Are records maintained of the radiation exposure of all employees who are monitored?
    Are radioactive materials stored, monitored and disposed of properly? Are storage containers labelled?
    Are copies of local and national radiation protection codes available? Are their contents known to staff?
    What shielding is available in the department?
    Are fume cupboards used for work involving radioactive vapours?
    Are detectors provided and periodically calibrated?
    Do staff know what to do about a radioactive spill?

Part 2. Microbiology

1. Is there a Safety Officer (infection)? Has he received any training?
2. Is there any attempt to train the staff in microbiological safety?
3. Are the office, washing up and domestic staff under the sole supervision of the Head of Department, or are they answerable only to other hospital officers, e.g. administrative staff or domestic superintendents?
4. Does the office staff actually handle specimens? Is there a distinction between clerical and reception staff? And a physical barrier?
5. What do the reception/office staff do with leaking or messy specimens? How is packing disposed of?
6. In what state do specimens arrive in the laboratory? Is there a safe method of transporting them from wards etc?
7. Who packs cultures, etc. sent to other laboratories by post etc? How is packing done?
8. Who supervises non-technical staff in the laboratory? Are these staff warned of the hazards of working in the laboratory?
9. What access do other persons, hospital staff, domestic, window cleaners, engineers etc. have to the working areas? Are they supervised while in the laboratory?
10. Is there a separate room for handling tubercle work? Who has access? Is any work with sputum done on the open bench?
11. Is there a microbiological safety cabinet? What make and year? Does it conform to current standards? How often is the airflow tested and by whom? Are the filters obviously dirty?
12. Is the laboratory obviously overcrowded with people and apparatus? What is the notional cubic floor space per person?
13. Are there hand basins in the laboratory rooms? What arrangements exist for changing or providing towels?
14. How often are overalls changed? Are those worn at the time of audit in reasonable condition? Is special protective clothing provided for high risk work?
15. Where do the staff put their outdoor clothes?
16. Is there a staff room? Do the staff wear their laboratory coats in the staff room or canteen? Is there any evidence of eating, drinking, smoking in laboratory rooms? Or any food in laboratory refrigerators?
17. Is there any evidence of known unsafe practices, e.g. mouth pipetting, culture plates piled too high, long, poorly made loops, poor racking, slide catalase tests, failure to use sealed centrifuge buckets for sputum and hepatitis specimens?
18. How are the cultures disposed of in the laboratory? In disinfectant, in wire crates, in boxes, bags, or in solid bottom containers?
19. What do they do with them in the wash-up room? Autoclave, incinerate? If material is incinerated is it autoclaved first? Who does the incineration — laboratory staff? Are cultures, discard material, left unattended anywhere, overnight, accessible to general public?
20. Are the autoclaves tested with thermocouples? By whom and how often? Are Brown's tubes or autoclave test strips used?
21. How often is the disinfectant in the discard jars changed? Is it the correct disinfectant for the work? Is there a hospital disinfectant policy?
22. Is an accident book kept? Are incidents that do not cause injury but which might have resulted in infection recorded?
23. Is a doctor available or accessible? Is the drill for wounding, e.g. with hypodermic syringe known to (a) laboratory, (b) hospital staff?
24. If a member of the staff is ill is there any check on possible laboratory infection? Does this apply to itinerant staff, e.g. porters who bring specimens, domestics who clean laboratories after normal working hours?
25. Do members of the staff inform their general medical practitioners that they are exposed to pathogens? Do they carry a card with this information and the telephone no. etc. of a medical officer of the laboratory or hospital??
26. Is there a protective inoculation policy? Are people who work with tubercle bacilli and new members of the staff given Mantoux tests and annual X-rays?
27. Is a stock of pathogenic organisms other than those habitually isolated, kept in the laboratory? Under whose supervision?
28. What are the general security arrangements against intrusion and vandalism?

Books on Laboratory Safety

Steere, N. V. ed Handbook of Laboratory Safety. Cleveland, Ohio: Chemical Rubber Company. A safety officer's bible.
Everett, K. and Hughes, D. (1975) A Guide to Laboratory Design. London: Butterworth. Larger teaching institutions will find this book very useful.

YOU CAN JUDGE US BY THE COMPANIES WE KEEP...

From August 1, McGaw Ethicals will no longer manufacture IV solutions, freeing the New Zealand operation to concentrate on marketing the most modern and innovative products available from its international group, American Hospital Supply Corporation and from other major suppliers.

The companies we will represent:

- American ACMI
- American Airlife
- American Edwards
- American Convertors/Tower
- American Critical Care
- American Heyer Schulte
- American Medical Systems
- American V. Mueller
- American Pharmaseal/Kormed
- American Scientific Products
- AHS/Australia Pty. Limited
- Atlantic Antibodies
- Axiom
- American Dade
- Cobe Laboratories
- Diagnostic Reagents Ltd. U.K.
- C.B. Fleet
- Disposable Products Pty. Ltd. Australia
- Exogen Limited U.K.
- Immulok
- General Diagnostics
- Gilson Medical
- Green Cross Corporation
- Harleco
- Marine Colloids
- Med-Kjemi
- Merz and Dade
- Nuclear Medical Laboratories U.S.A.
- Organon Teknika
- Parker Laboratories
- Quinton Instruments
- S.M.I. – U.S.A.
- Sentry Medical
- Tecnol

McGaw Ethicals Ltd.

P.O. Box 18-069.
Auckland 6. New Zealand.
Subsidary of American Hospital Supply Corporation.

Muri, G. D. ed Hazards in the Chemical Laboratory. London: Royal Institute of Chemistry. "Muir" has its rightful place in most chemical laboratories but is rarely seen in pathology laboratories. This is wrong.
Howe, J. R. (1975) A method of recognising carcinogens in the laboratory. Laboratory Practice, 24, 457. This paper offers a classification system for identifying and avoiding carcinogens that is most useful for laboratory workers.
Imperial College of Science and Technology (1974) Precautions against Biological Hazards. London: Imperial College. For biologists this is a 'best buy'.
Collins, C. H., Hartley, E. G. and Pilsworth, R. (1975) The Prevention of Laboratory Acquired Infection. Public Health Laboratory Service Monograph Monograph No. 6 London: HMSO. Intended for use in laboratories where pathogenic organisms are handled.
Everett, K. and Jenkins, E. W. (1973) A Safety Handbook for Science Teachers. London: John Murray. An excellent book: the information is presented with admirable economy of words and a typographical excellence which ensures its impact on the reader.
Safety in Laboratories (1974). London: Ciba-Geigy (UK) Limited. This is a useful small booklet of the does and don'ts for chemical laboratory workers and is intended to form a basis for a code of practice for laboratory safety.
Department of Education and Science. Safety in Science Laboratories. London: HMSO. A great deal of useful information is contained in the forty pages of this booklet.
Safety in Universities: Code of Practice. Part 1 — General Principles. The Committee of Vice-chancellors and Principles of the Universities of the United Kingdom, 29 Tavistock Square WC1H 9EZ. This booklet provides background information and general principles of the administration of safety organization.
UMIST Safety Manual. The University of Manchester Institute of Science and Technology, P.O. Box 88, Manchester M6O 1QD. Most university science departments have safety manuals, but this is the best I have seen.
Department of Health and Social Security (1972) Safety in Pathology Laboratories. This is supposed to be issued to every person who works in an NHS Department.
Royal Institute of Chemistry (1976). Code of practice for Chemical Laboratories. Essential for chemists. Most useful for other laboratory workers.
DHSS (1979) A Code of Practice for the Prevention of Infection in Clinical Laboratories and Post-Mortem Rooms. London: HMSO. The standard microbiological safety work in the UK. The 'Howie' Code.
Collins, C. H. (1983) Laboratory Acquired Infections. London: Butterworths. History, incidence, causes and prevention.
World Health Organisation (1983) Laboratory Biosafety Manual. Geneva: WHO. Rivals the Howie Code. Better for research laboratories.

CSU NOTES

Annual Holidays Act

1. The following letter of 9 June has been received from the Department of Labour:—

"You may remember that last Christmas the New Zealand Dairy Workers Union threatened to withdraw their labour on 25/26 December and 1/2 January unless changes were made to the Holidays Act to rectify what they considered to be an unsatisfactory state of affairs regarding payment for work on these statutory holidays when they fall on weekends.

The Holidays Act 1981 provides that where (for example) Christmas Day falls on a Saturday then provisions governing work on a Saturday shall apply without modification and for purposes of pay and observance of a holiday Christmas Day shall be transferred to the following Monday. Similar provisions govern the observance of Boxing Day, New Year's Day and 2nd January.

The transference of statutory holidays in this way ensures that persons who work a normal Monday to Friday week receive a paid holiday for the statutory holidays concerned. In this respect it works satisfactorily but it does disadvantage those workers who normally work on a Saturday or Sunday but not on a Monday or Tuesday. When these workers work on a Saturday that coincides with Christmas Day they are eligible only for Saturday payments and because they do not normally work on Monday (the day the holiday is transferred to) they are not entitled to special conditions associated with working on the statutory holiday.

At the time the parties were seeking an amendment to the Holidays Act the Minister of Labour responded to the effect that the issue is one that affects other industries in addition to the dairy industry and that the issue should be widely canvassed. The Minister undertook to have the matter investigated with the aim of rectifying the problem prior to Christmas 1983 when it will again arise in a modified form.

The Department has developed a proposal. In essence it would provide that where a worker works (as part of his normal work week) on a Saturday that also happens to be Christmas Day **and** he does not work on the following Monday (the day the holiday is normally transferred to) then for that worker the provision governing rates of pay for work on Christmas Day shall apply on the day the holiday actually falls.

The same principle would apply in respect of work on Boxing Day, New Year's Day and 2nd January.

Specifically, we would see the proposal as an amendment to the Holidays Act to be inserted as S.9(4).

Subsections (1) to (3) of this Section shall not apply where a worker:

(a) Actually works on a Saturday or Sunday being Christmas Day, Boxing Day, New Year's Day or the second day of January, as the case may be; and

(b) Does not actually work on the following Monday (in the case of the holiday falling on a Saturday) or Tuesday (in the case of the holiday falling on a Sunday) —

and in such a case any provisions governing payment of wages or other conditions of employment on Christmas Day, Boxing Day, New Year's Day or the second of January, as the case may be, shall be construed as applying on the day the holiday falls.

An amendment along these lines could be limited to the dairy industry or it could be enacted to have wider application as we are aware that the problem has arisen in other industries.

In the latter case the amendment would have implications for the state sector. For this reason the Department is seeking the views of your organisation on the proposal and its possible scope. The Department is also consulting with central organisations in the private sector on this proposal and also on the standing of the Holidays Act vis a vis the Industrial Relations Act.

State Pay Fixing

At the meeting with the Cabinet/Caucus Committee on 18 May the government representatives gave no indication of taking into account any of the points made to them at the meeting in April and it is clear that the Government intends to proceed with changes to the pay fixing legislation which have the main aim of economising on state employees. The government representatives suggested that there could be some discussion of alternatives with officials but the CSU have responded to the effect that it sees little point in such discussions without a clear policy direction from the Government as to its intentions on each of the proposed changes. This would require a discussion on a clause by clause basis with the Cabinet/Caucus Committee before matters of detail were discussed with officials.

In the meetings in the five main centres the CSU speakers have underlined the danger to the pay of state employees inherent in the Government's proposals and the departure from fair relativity principles that these changes constitute.

Anzac and Waitangi Days

The SSCC gave advice of its wish to amend determinations in the Public Service, Hospital Service and Broadcasting Service to bring them into line with less favourable conditions in other services and in the private sector. The CSU responded to the effect that in current circumstances it was not prepared to discuss any changes to determinations.

THE PACIFIC WAY

Each issue of the N.Z.I.M.L.T. Journal will in the future contain some information relating to Laboratory Technology in the Pacific area, on a page entitled "The Pacific Way". Initially it will bring you up to date with news about the P.P.T.C., people and personalities involved in Medical Laboratory Technology in the Pacific, and general items about individual island laboratories. In the long term in "The Pacific Way" in "Pacific Time" it is anticipated that items of interest and articles from Pacific Island laboratories will appear on these pages.

Pacific Time always implies that some things will take place somewhat later than planned, or at least later than formally stated and that in extreme cases they may not necessarily take place at all.

The term "The Pacific Way" was launched on the international stage by Fiji's Prime Minister, Ratu Sir Kamisese Mara in an address to the United Nations General Assembly in 1970. Dr. Ron Crocombe, Professor of Pacific Studies at the University of the South Pacific, Suva, Fiji, in his book "The Pacific Way — an emerging identity" states: "It was a term born of political necessity in that it helped to fulfil a growing demand for respected Pacific-wide identifying symbols and Pacific Unity. Since 1970 the term "The Pacific Way" has been widely used. It has no precise meaning. Its great advantage is it's built in flexibility. It is totally non-specific, so that whatever the reality of the particular time, place or context within the Pacific almost any activity can be classified as a manifestation of the Pacific way".

Pacific Paramedical Training Centre

The Centre is now into its third year of operation. From April 1982 until June 1983 a total of 18 trainees completed courses at the P.P.T.C.

Trainees who Completed Courses at the Pacific Paramedidical Training Centre April 1982-June 1983

Mr Obeta Tioti (Red Cross Health Science Award) Honiara, Solomon Islands. Immunohaematology April-June 1982., Mr Vijendra Prasad (Red Cross Health Science Award) Lautoka, Fji. Immunohaematology April-June 1982., Miss Ruth Reeves (VSA Candidate) Orientation Course — Pacific Island Medical Laboratory Technology. February 1982., Mr Chris Simmons (Private) Orientation Course — Basic Microbiology for Developing Countries. June-July 1982., Mr Ansuiya Devi Singh (WHO Fellow) Lautoka, Fiji. Water and Food Technology Course Sept.-Dec. 1982., Mr Sitirio Maka (WHO Fellow) Nuku'alofa, Tonga. Water and Food Technology Course Sept.-Dec. 1982., Mr Semiperieve Ieremia (WHO Fellow) Apia, Samoa. Water and Food Technology Course Sept.-Dec. 1982., Mr Ngatokorua Teariki (WHO Fellow) Rarotonga, Cook Islands. Water and Food Technology Course Sept.-Dec. 1982., Mr Florencio Yamada (WHO Fellow) Palau, TTPI. Water and Food Technology Course Sept.-Dec. 1982., Mr Morsou Takju (WHO Fellow) Majuro, TTPI. Water and Food Technology Course. Sept.-Dec. 1982., Mr Baibuke Tauro (WHO Fellow) Tarawa, Kiribati. Water and Food Technology Course Sept.-Dec. 1982., Mr George James (Award — N.Z. Country Women's Institute) Madang, Papua New Guinea. Water and Food Technology Course Sept.-Dec. 1982., Mr Alan Pipi (N.Z. Government Bilateral Aid Programme) Port Moresby, Papua New Guinea. Water and Food Technology Course Sept.-Dec. 1982., Mr Chris Simmons (Private) New Zealand. Water and Food Technology Course Sept.-Dec. 1982., Mr Vaevaetaearoi Pare (Red Cross Health Science Award) Rarotonga, Cook Islands. Immunohaematology March-May 1982., Mr Kirisimasi Poasa (Red Cross Health Science Award) Apia, Western Samoa. Immunohaematology March-May 1982., Mr Estras Sumor (WHO Fellow) Blood Bank/Haematology March-May 1982., Miss Areta Aritiera (N.Z. Government Bilateral Aid Programme) Tarawa, Kiribati. Blood Bank/Haematology March-May 1982.

Facilities and Equipment

Thanks to the generosity of a number of groups, the equipment in the Teaching Laboratory has continued to improve steadily over the past year. In addition to the many pieces of equipment which were donated to the Centre by individual laboratories, the acquisition of the overhead projector and new constant temperature waterbath were of particular note. The budget on which the Centre operates still remains very limited, however, and as the scope of the courses taught increases, there will be little money available for any additional equipment required.

In expressing our gratitude to all who have assisted us in the past, we look forward to your continued support.

P.P.T.C. Students & Staff, Water Course, 13th Sept.-2nd Dec. 1982. Students left to right (back row) Baibuke Tauro (Kiribati), Morson Takju (T.T.P.I.), George James (Papua New Guinea), Florencio Yamada (T.T.P.I.), Ansuiya Devi Singh (Fiji), Chris Simmons (N.Z.), Alan Pipi (Papua New Guinea), Semiperieve Ieremia (W. Samoa), Sitirio Maka (Tonga). Left to right — (front row) Dr. H. C. Ford, (Co-chairman, P.P.T.C.), Alison Redman (Project Employment Person), Andrea Hall, Tutor P.P.T.C., Ron McKenzie (Co-chairman P.P.T.C.).

The Minister of Health, the Hon. A. G. Malcolm presenting certificate to Morson Takju from Majuro, T.T.P.I. at the completion of the Water Course. In the background are the two Co-chairmen of the P.P.T.C. Committee, Dr. H. C. Ford and Mr Ron McKenzie.

Visitors to the Centre 1982-83

Hon. A. G. Malcolm, Minister of Health who presented certificates to the trainees who completed the Water and Food Technology Course in December, 1982.

Dr. Keith Ridings, Assistant Director Hospitals Division, Department of Health presented certificates to trainees who completed courses in May of this year.

Dr. N. U. Rao, Laboratory Advisor, W.H.O. Fiji (two visits) and Dr. I. Geizer (one 3 day visit) Medical Officer for Laboratory Technology, W.H.O. Regional Office, Manila, have provided help, encouragement and stimulation to the course organisers.

In addition to these overseas visitors to the P.P.T.C. there have been a number of local people in various health fields who are interested in the concept of appropriate medical technology for developing countries. Andrea Hall and members of the Committee have also taken the

opportunity to speak to a variety of organisations to gain support for the Centre's work.

Letter from Cook Islands

The following is a letter from Vaevae Pare, a Senior Technician from the Cook Islands who attended the 3 month Blood Bank Course at the P.P.T.C. from March 7th-May 27th. Vaevae was sponsored by the New Zealand Red Cross. "Recently I have completed a 3 months course on Immunohaematology held at the Pacific Paramedical Training Centre at Wellington Hospital. The Course was attended by 4 participants selected from various laboratories in the Pacific. One each from Kiribati, Western Samoa, Island of Yap and myself from the Cook Islands. All 4 participants are laboratory technicians, who are trained locally in their respective laboratories and have limited understanding and knowledge of the mentioned subject. During the 3 months of the Course I was very much impressed by the way the course was held and how simplified the teaching was, which I think, suits our limited level of education and capabilities.

I have gained through this Course many experiences that are new to me of which I think will be of importance in improving our Blood Bank section. There are a lot of things that are taught in the course that are not being applied in our laboratory at present and I know a quick change will upset the present system, but there is surely a need for improvement.

On my return, I am very keen to share with my fellow technicians and trainees all my experiences gained in this 3 months course. The things that need to be changed and the new methods and techniques to be introduced. I hope the other participants in the course would be able or given the chance to do the same when they return to their laboratories.

I have found this short Pacific Paramedical Training Course very important and valuable for Pacific Island Laboratory technicians and a 3 months period is adequately sufficient for any such course. I also think that candidates who are attending the courses must be well selected and must have at least 2 years or more in their own laborotories. This will give them training and understanding of basic laboratory tests so as to meet the level and standards of teaching given at the Pacific Paramedical Training Centre.

There are other things that we participants could gain from the Pacific Paramedical Training Centre. There is unused laboratory equipment and apparatus donated to the P.P.T.C. laboratory from various N.Z. laboratories which are no longer of use to them. They would be of valuable use to several of our Pacific Laboratories. The P.P.T.C. is very willing to offer this equipment and apparatus to participants for their own use in their laboratories. I hope more equipment would be donated to the P.P.T.C. in the future so they could be offered to participants in future courses.

I would like to give my sincere thanks to those keen Organizations who supplied aid to sponsor participants from the developing laboratories in the Pacific to attend such training courses. I would also like to give my appreciation to laboratories in Masterton, Dannevirke, Palmerston North and Wellington Blood Transfusion Centre, for their assistance and help in the 3-4 weeks where each of us spent time during the term of the course. Lastly I would like to convey our appreciation to the Pacific Paramedical Training Centre and to our tutor, Andrea Hall, for making the course a valuable and successful one".

Thank you Vaevae for your comments.

Watch out for the Pacific Way in future editions of the N.Z.I.M.L.T. Journal.

Marilyn M. Eales

BOOK REVIEW

Man Made Life: A Genetic Engineering Primer. By Jeremy Cherfas. Published by Basil Blackwell. Publisher.
$16.50 paperback.
New Zealand distributor Benton Ross Publishers Ltd., P.O. Box 33-055, Takapuna, Auckland 9.

"MAN MADE LIFE" by Jeremy Cherfas is an unusually competent "popular" science book on the fascinating subject of genetic engineering. It is aimed at readers with a general scientific background but without special experience of genetics or of microbiology, the discipline within which the major portion of the work has been performed.

The reader is first conducted sympathetically but unpatronisingly through the background to the subject. The fascinating story of how the nature of genetic material was determined is succinctly reviewed, as are the methods by which the process of gene replication were determined.

The tools of the genetic engineer are now many. Perhaps the most indispensable ones are the restriction enzymes. Recognition that it was their action which prevented proliferation of certain bacteriophages within strains of bacteria which normally allow them to proliferate was fundamental. The determination of how restriction enzymes act and how they know where to act on the DNA of the phage is described in some detail. Their application to locate and cut DNA at predetermined points has led to their description as "precision scalpels". The role of other enzymes, terminal transferases, DNA polymerases, reverse transcriptases etc. is described, all contributing to the insertion of the base sequences determining the desired activity into the reconstituted gene.

With this background the reader is taken on an exhilarating trip through the principal techniques employed in genetic engineering. The principal requirements are a method of breaking and joining DNA from different sources, a gene carrier that can replicate itself and a foreign segment incorporated therein a method of getting the composite molecule into a functioning bacterial cell and a method of selecting a clone that has acquired the molecular chimera. The manner in which these requirements have been achieved is then outlined.

Manipulation of the genetic character of living organisms carries disturbing overtones. The way in which potential hazards were regulated and evaluated, largely by members of the discipline itself is outlined. How real were the fears concerning possible catastrophic escape of hazardous mutants? The subject is discussed with particular reference to the current phase in which the techniques are moving, or have already moved, from the laboratory to the factory. The vast financial advantages that are beginning to appear may carry the possibility of distortions of judgment.

However the immense potential for good, even in the short term is tremendous. Insulin produced by genetic manipulation of bacteria is already being marketed. Interferon is on the threshold. Hormones, vaccines, for veterinary as well as human use, are imminent, some of them of an entirely novel character. It is even possible to contemplate the modification of genetically determined states such as sicklecell anaemia or Downs Syndrome.

The author treats all this in such a manner as to produce a highly readable book. I would not say I couldn't put it down but I certainly had no trouble in picking it up at frequent intervals until I had finished its 250 odd pages.

E. G. McQueen

INSTITUTE BUSINESS

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Biochemistry Dept., National Women's Hospital, Auckland.

Membership Secretary
Margaret Young
Laboratory, Waikato Hospital, Hamilton.

Membership Fees and Enquiries

Membership fees for the year beginning April 1, 1983 are:
For Fellows — $40 reducible to $35 if paid by June 30 that year.

For Associates — $40 reducible to $35 if paid by June 30 that year.

For Members — $30 reducible to $25 if paid by June 30 that year.

For Non-practising Members — $20 reducible to $15 if paid by June 30 that year.

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.

THE NEW EDITOR

Introducing DENNIS DIXON-McIVER, the new Editor.

I have recently been appointed Editor of the Journal to succeed Hugh Matthews and below is a short profile.

I am aged 34, married with two children. I commenced training in 1967 and trained at North Shore Hospital and Princess Mary Laboratory, Auckland and qualified in 1971. I was a staff technologist in the Biochemistry Department, Princess Mary Laboratory 1972-74 and then was Graded Officer Second-in-Charge, Biochemistry Department, Princess Mary Laboratory 1974-80. I was appointed Charge Technologist, Biochemistry Department, National Women's Hospital in 1980. I was a committee member of the Auckland Branch in 1982, and Chairman in 1983.

My main interests are sport, trotting, reading and education. I am a member of my children's school P.T.A. and school committee.

The production of the Journal is very much a team effort and my assistants are Raewyn Bluck, Bruce Dove, Ian Green, Dennis Reilly, Mary Sorenson and Walter Wilson and valuable secretarial assistance is being given by Jo Caddy. The **advertising manager** is Trish Reilly, 48 Towai Street, St. Heliers, Auckland, 5, ph: 555-047.

Previous Editors have continually made pleas to the members for contributions and I will reiterate their pleas. The Journal is dependent on members for its continued existence, so please make the Editor's job easier by contributing original articles for publication.

BRANCH NEWS

DUNEDIN BRANCH

The office bearers 1983-84 for the Dunedin Branch NZIMLT are as follows:—

Chairman — Mr Zigmunt M. Poczwa
Secretary — Mrs Jan Parker
Treasurer — Mrs Christine Pickett
Committee — Miss Jan Deans
Miss Julie Peters
Mrs Debbie Sloper

CHRISTCHURCH BRANCH

The Annual General Meeting of the Christchurch Branch of the NZIMLT was held at Christchurch on June 13, 1983. The following officers were elected:—

Chairman — Kathleen Ahern
Secretary — Christine Hickton
Treasurer — Geoff Mills
Committee — Brett Hobson
Howard Potter

LETTERS TO THE EDITOR

Dear Sir,

re: **"Manufacturers Reference Ranges"**

Despite reagent kit manufacturers suggestions that each Laboratory establish its own Reference Ranges, particularly for enzyme analysis, it is frequently assumed that the manufacturers have in fact selected an appropriate healthy population and established a "correct" reference range. A new ALT method was recently introduced into this laboratory and a reference range study conducted for ALT along with a number of other analyses. All except the ALT confirmed the manufacturers range. For ALTs the following was found for a population of blood donors.

n = 92 (49 male, 43 female)
distribution was Gaussian
mean 11.6
SD 4.0
Range 3-20 IU/l at 30°C
One outlier was present, a donor with obvious liver disease.
The manufacturers quoted range at 30°C was: Males 0-47 IU/l
Females 0-36 IU/l

An enquiry was directed to the manufacturers and the following telex reply received.

"Agree, GOT/GPT ranges unusually high. Best that each lab establish its own range. We are setting up to reestablish the range with a different population."

That reply says all!

Tony Smale
Oamaru

Dear Sir,

The Medical Group of Amnesty International, New Zealand Section was formed in June 1982, following the publication of a letter in the NZ Medical Journal, appealing for members. Since then the numbers have grown to thirty four, of whom twenty five take part in Medical Letter Writing Actions. Since it is now a year old, it seems timely to circulate a brief review of its activities to members.

The Medical Group is open to doctors, nurses and paramedical professionals. At present thirty one of its members are registered medical practitioners. It is hoped that increased publicity to other groups will bring in more of them as members. Letters are being sent to professional journals and it is hoped to arrange meetings in the larger hospitals during the rest of the year.

Medical Letter Writing Actions (MLWAs) directed to the Medical Group are concerned with prisoners of conscience who are suffering from disease and receiving inadequate, or no, medical treatment, with prisoners who are, themselves members of the medical and related professions and with issues such as the involvement of doctors at torture centres and in executions.

Six copies of MLWAs are sent to the medical co-ordinator as they arise. At present more appeals are received than can be distributed but it is hoped this will improve as membership increases. Members are asked to state the interval at which they wish to receive MLWAs and care is taken that they are not sent them more frequently.

A letter on behalf of the group, appealing for the medical profession to join in letter-writing action, concerning the Amnesty Report: Chile: evidence of Torture, will appear in the NZMJ for June 8. A request has been made to the NZMA to express their disapproval of doctor's presence at torture sessions to the Colegio Medico de Chile. A letter, on behalf of the group, has been sent to the Colegio Medico in addition to letters from individual group members to it and other official bodies.

Members ideas for extending the work of the group and any views on the way it is run will be welcome.

I should like to thank all members for their support and appeal to them to make the existence and work of the Medical Group known to their colleagues.

Jill Bailey Medical Co-ordinator
279 Bleakhouse Road,
Howick,
Auckland.

NEW PRODUCTS AND SERVICES

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Four operating keys on the keyboard enable the user to call up all functions of the DU-6 Spectrophotometer. The "single λ" key facilitates simple absorbance or % transmission measurements at one wavelength by calculating answers and displaying results on the integral video display. The "multi λ" key reads up to eight wavelengths and has the optional ability to calculate quantitative answers for samples that contain up to five different components. For routine wavelength scanning spectra in absorbance, % transmission and first or second derivative, the "λ scan" key takes sample and reference scans at exactly the same wavelengths, automatically performs baseline correction and eliminates tracking errors. The "time drive" key accommodates all time-dependent measurements in absorbance or % transmission,

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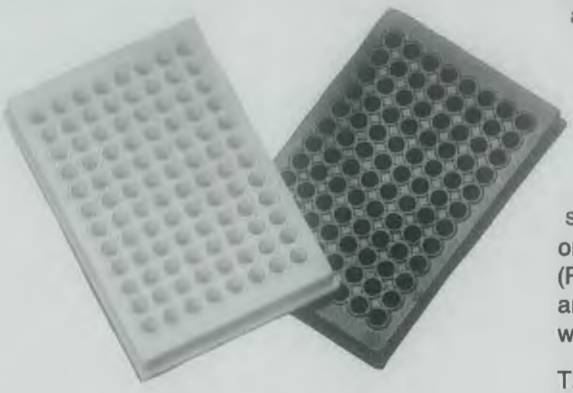

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ABSTRACTS

MICROBIOLOGY

Diseases of the Fetus and Neonate due to Human Cytomegalovirus: A Library Perspective.
Tyms, A.S. (1982), *Med. lab. Sci.* **39,** 275.
This is a review article on human cytomegalovirus and includes a brief description of the present laboratory diagnosis and what future developments might be.

Rapid Trichrome Stain.
Flournoy, D. J., McNabb, S. J. N., Dodd, E. D. and Shaffer, Marilynne H. (1982), *J. clin. Microbiol.* **16,** 573.

A rapid trichrome stain is described, using the same reagents as the Wheatley trichrome method but putting them on slides dropwise, then volatilising them with a slide warmer and drying the slides between steps. The stain was tested on smears containing *Giardia lamblia, Entamoeba histolytica, E. Nana, E. hartmanni, I. butschlii,* and *C. mesnili* trophozoites and cysts and *D. fragilis* trophozoites. It compared well with the conventional trichrome stain.

Determination of Bacterial Meningitis: A Retrospective Study of 80 Cerebrospinal Fluid Specimens Evaluated by Four In Vitro Methods.
Wasilauskas, B. L. and Hampton, K. D. (1982), *J. clin. Microbiol.* **16,** 531.

The four tests used on the CSF specimens included routine culture, gram stain, countercurrent immunoelectrophoresis (CIEP) and Staphylococcal coagglutination (CoA) with laboratory prepared reagents and Pharmacia Diagnostics reagents. All CSF specimens were tested for antigens of *H. influenzae* type b, *S. pneumoniae* (all 83 capsular types) and group B streptococci by the 3 immunological tests. CSF was also tested for *N. meningitidis* types A-D, X, Y, Z by CIEP and laboratory CoA but the reagents were not available for the Pharmacia CoA. 60 out of 80 specimens of CSF were positive for meningitis by one or more of the methods. Cultures were positive for meningitis by one or more of the methods. Cultures were positive in 56 specimens but gram stains were positive in only 36 of these specimens. The four specimens that had negative culture results were positive by one of the antigen detection methods, the CoA tests being more sensitive than CIEP. Not all the culture positive specimens were positive by the immunological methods and a false positive result was obtained with each of the *Strep. pneumoniae* antisera for a CSF that grew *K. pneumoniae*. The authors recommend that immunological methods be used as an adjunct to the gram stain and culture in the diagnosis of meningitis.

Comparison of Two Methods for Same-Day Identification of Enterobacteriaceae.
Applebaum, P.C., Arthur, R. R., Parker, M. E., Shugar, G. L., Von Kuster, L. C. and Charache, P. (1982), *Am. J. clin. Pathol.* **78,** 351.

The two methods evaluated were API—20E at 5 hours and Micro-ID at 4 hours and organisms included all the Enterobacteriaceae. The API-20E identified 78.5% of strains to species level and 9.5% to genus only. Micro-ID identified 90.0% to species level and 3.3% to genus level only. The Micro-ID was easier to inoculate and read whereas with the API-20E system the sugar fermentations and decarboxylase/dihydrolase/deaminase reactions were not always clear cut. Many of the Serratia, Citrobacter and Providencia strains were only identified to genus level with the API—20E and *Serratia marcescens* gave the most incorrect results with both systems.

Rapid Biochemical Identification of Gram Negative Bacilli from Blood Cultures Using API-20E Strips.
Furtado, G. L. and Medeiros, A.A. (1982), *Am. J. clin. Pathol.* **78,** 356.

3ml of blood culture broths containing gram negative bacilli were washed with cold sterile water and the deposit suspended in normal saline to inoculate API-20E strips. Of 126 blood culture isolates tested 100% were speciated correctly.

Shirley Gainsford

HAEMATOLOGY

Platelet Aggregation in Whole Blood Determined Using the Ultra-Flo 100 Platelet Counter.
Fox, S. C., Burgess-Wilson, M., Heptinstall, S. and Mitchell, J. R. A. (1982), *Thrombos Haemostas (Stuttgart)* **48,** 327.

Most studies of platelet aggregation are carried out in platelet-rich plasma which has been derived from anticoagulated blood by removal of the bulk of red and white cells. This is because light transmission techniques for semi-quantitation of platelet aggregation in platelet-rich plasma are readily available. The authors suggest that whole blood techniques would be preferable because in platelet-rich plasma a population of hyperactive platelets may be lost in preparation. The authors detail a technique of whole blood aggregation using a commercial platelet counter.

An Automatic Method for Thrombotest on the "Cobas Bio" Centrifugal Analyser.
Karlsen, R. L., Visdal, A. and Stensberg, A. (1982), *Scand. J. clin. Lab. Invest.* **42,** 647.

The thrombotest assay was adapted by the authors to a centrifugal analyser and they found excellent correlation to results obtained on a coagulometer. When linked to a computer, the method reduced manual labour and time by 50%.

Immune thrombocytopenias and Platelet Antibodies.
Moore, S. B. (1982), *Mayo Clin. Proc.* **57,** 778.

The author gives a brief review of immune thrombocytopenias, including tests for platelet antibodies.

Microcytosis: Its Significance and Evaluation.
Steinberg, M. H. and Dreiling, B. J. (1983), *JAMA* **249,** 85.

Smaller-than-normal red cells (microcytosis) are commonly detected as a by-product of red counts measured by electronic particle counters. The differential diagnosis of microcytosis is discussed and an approach to its evaluation is outlined.

Erythrocyte Ferritin Content in Idiopathic Haemochromatosis and Alcoholic Liver Disease with Iron Overload.
van der Weyden, M., Fong, H., Salem, H. H., Batey, R. E. and Dudley, F. J. (1983),*British Medical Journal.* **286,** 752.

The erythrocyte ferritin content was measured in patients with idiopathic haemochromatosis and alcoholic liver disease with iron overload to define its value as a marker for an excess of tissue iron. The mean erythrocyte ferritin content in patients with untreated haemochromatosis was increased 60 fold and fell with phlebotomy. After phlebotomy any patients had an increased red cell ferritin content despite normal serum ferritin concentrations. The mean erythrocyte ferritin content in patients with alcoholic liver disease and iron overload was increased only 7 fold and the ratio of serum ferritin to erythrocyte ferritin clearly discriminated these patients from those with idiopathic haemochromatosis.

Estimation of Lymphocyte Percentage and Number on the Coulter Counter, Model S Plus Phase II.
England, J. M., Chetty, M. C. and DeSilva, P. M. (1982), *J. clin. Pathol.* **35,** 1194.

The Coulter Counter Model S Plus Phase II provided precise measurements of lymphocyte percentage and count and carryover was negligible. Lymphocyte percentage values agreed well with those from the stained film except when the percentage was high and in these circumstances the instrument gave underestimates. The display of leucocyte volume distribution was found to be a useful attribute particularly when the instrument alerted the operator to "rejected" profiles.

A Rapid Screening Method to Increase Efficiency in Assaying Plasma Levels of Clotting Factor VIII:C Imhibitors.
White, K. S., Dombrose, F. A. and Blatt, P.M. (1982), *A.J.C.P.* **78,** 450.

The authors describe a screening test for inhibitors based on the percentage of Factor VIII:C remaining in a normal plasma control after incubation with the Factor VIII:C inhibitor for 2 hours at 37°C.

E. R. Crutch

HISTOLOGY

Fine Needle Aspiration Biopsy.
Frabile, W. J. (1983), *Human Pathology.* **14,** 9.
A comprehensive review article.

Oestrogen Receptor Assay of Cryostat Sections of Human Breast Carcinomas with Simultaneous Quantitative Histology..
Underwood, J. C. E., Dangerfield, V. J. M. and Parsons, M. A. (1983), *J. Clin. Path.* **36,** 399.
Cryostat sections of unfixed human breast carcinomas were assayed for oestrogen receptor content. Adjacent sections were stained so that tumour content could be estimated. Elastosis was also assessed.

A Comparative Study of Generic Stains for Carcinoid Secretory Granules.
Smith, D. M. and Haggitt, R. C., *Am. J. Surg. Path.* **7, 1,** 61.
A study which sets out to determine which method most consistently demonstrates carcinoid granules. A battery of six stains is applied to 73 tumours.

Protargol Silver Impregnation and Neuronal Selectivity.
Zagin, I. S. and Haring, J. H. (1982), *Acta. Anatomica.* **114, 3,** 193.
Light and Electron microscope observations are presented. The authors experiences in using this 'notably capricious' technique are discussed, and emphasis is placed on the necessity for correct fixation.

Factor VIII Related Antigen and Mast Cells..
Kindblam, L. G. (1982), *Acta Pathologica et Microbiologica et Immunologica Scandinavica* **90, 6,** 437.
From an immunoperoxidase technique used to demonstrate Factor VII related antigen, it was observed that human mast cells were positively stained in observed areas. This is considered to support the opinion that mast cells may play a role in local haemostasis.

New Developments in Immunoperoxidase Techniques and their Application.
Falini, B. and Taylor, C. R. (1983), *Arch. Pathol. Lab. Med.* **107,** 105.
Immunoperoxidase methods are now used extensively in surgical pathology. Many variations of the technique have been developed since the basic immunoperoxidase method was first applied 'routinely' in 1974. A special review article which accounts the particular strengths and weaknesses in each variation.

Mechanical Embossed Labeling of Plastic Cassettes for use in Histology and Pathology Laboratories.
Schoepfer, A. (1983), *Human Pathology* **3,** 278.
An automated embossing machine for tissue Tek-type cassettes is described.

A Staining Procedure for Melanin in Semithin and Ultrathin Epoxy Sections.
Duinen, S. G. van., Ruiter, D. J. and Scheffer, E. (1983). *Histopathology.* **7,** 35.
A modified Warthin-Starry procedure for melanin identification is described. It may act as an important diagnostic aid by helping identify apparently amelanotic melanomas.

A New Staining Method for Legionella pneumophilia.
Tseng, C. H. and Renner, E. D. (1983), *Am. J. Clin. Path.* **9,** 377.
This report describes a method which seems to consistently stain *L. pneumophilia* in tissue sections. Its advantages over other methods currently in use are discussed.

Acid Esterase Activity in Lymphocytes and Other Cells: A Comparison of Six Alpha-Naphthyl Based Substrates.
Freemont, A. J. and Davies, J. S. (1982), *Med. Lab. Sci.* **39,** 405.
An investigation into the use of alpha-naphthyl based substrates to improve stain density using shorter incubation periods in order to aid the differentiation of the characteristic granules identifying T. lymphocytes.

B. C. Thackeray

BIOCHEMISTRY

Is Routine Maternal Serum A-Fetoprotein Testing a Waste of Time in an Area of Low Incidence of Neural Tube Defects?
Mary J. Seller. *J. Obstet & Gynaec.* (1983) **3,** 139.
All patients referred for amniocentesis because of one or two raised maternal serum a-fetoprotein levels in an area of low incidence of neural tube defects in the south-east of England were studied at amniocentesis and at the outcome of pregnancy, and compared with women undergoing amniocentesis for other reasons. Ten per cent of women with two raised serum a-fetoproteins had fetuses with neural tube defects, half of which were spina bifida. Four per cent had other serious fetal conditions and eight per cent had intra-uterine deaths or spontaneous abortions. Only 74 per cent had a live born baby without abnormality, and these tended to be male and to weigh less than average.
Neural tube defects were not found to any large extent in the women with only one raised serum a-fetoprotein level, emphasising that two raised levels are a far better predictor for neural tube defects. The former group resembled the latter at delivery with regard to an excess of low birth weight babies and preponderance of males.
It is beneficial to identify women with raised serum a-fetoprotein levels in a low neural tube defect incidence area, not only from the point of view of diagnosing these defects, but also for detecting other serious fetal conditions and designating a high risk group for fetal loss.

Laboratory Diagnosis of Cystic Fibrosis
H. Lewis Webster. *CRC Critical Reviews in Clinical Laboratory Sciences,* **18, 4,** 313.
The demonstration of abnormally high concentrations of electrolytes in eccrine sweat is still the only practical laboratory procedure available for diagnosis of cystic fibrosis. Properly performed, the sweat test is very reliable, but there are many published reports that all of the methods in current use frequently generate incorrect diagnoses. Analysis of potential for error in sweat test methods shows that of the three essential phases involved, stimulation, collection and analysis, the major cause of intrinsic inaccuracy occurs in the collection process. In this case the problem is due to condensate formation, which leads to the subsequent analysis of nonrepresentative sweat. Human error is also an important cause of false results and is a direct function of the number of critical manual operations involved in the technic. This review provides a critical examination of sweat test methods, identifying problem areas and suggesting ways to improve procedures in the interests of clinically reliable laboratory data in support of diagnosis.

Performance of the Hitachi 705 Evaluated
Pierre Douville and Jean-Claude Forest. *Clin. Chem.* **vol. 29,** 4, p.692 (1983)
We evaluated a new discrete analyzer, the Hitachi 705. The instrument can process sequentially as many as 19 different enzymes or other analytes, with a throughput of 180 tests per hour. We evaluated the spectrophotometer, the sample and reagent probes, and the performance for 11 assays: creatine kinase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyltransferase, amylase, glucose, urea nitrogen, creatinine, albumin, calcium and phosphorus. The total imprecisions (within-day plus day-to-day component of imprecision) (CV) were:— for the photometer (six concentrations of potassium dichromate), 0.14 to 0.4%: for the serum probe (55 to 20 μL), 0.4 to 1.0%: for the reagents probes (50 to 350 μL), 0.3 to 1.2%: for the enzymatic procedures, 2.0 to 8.0%; and for the other tests (glucose, urea nitrogen, creatinine), 1.0 to 4.0%. Good linearity was obtained in the proposed range for the 11 tests, except for calcium. Comparison of the total analytical error with the medically allowable error at a decision value showed that the test results were acceptable, except for calcium and phosphorus. The instrument showed good reliability and practicability.

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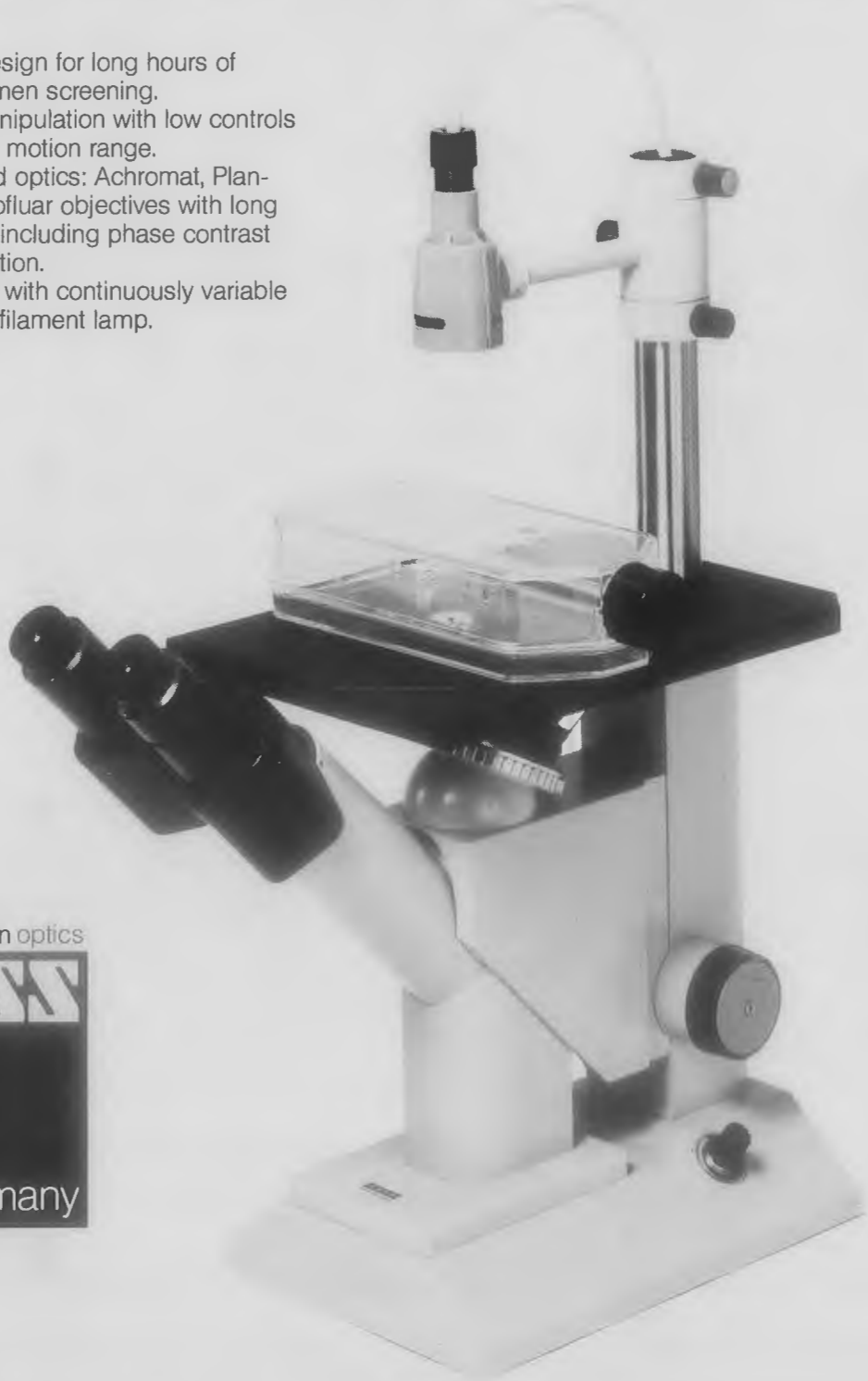

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