JULY, 1979

ISSN 0028-8349

THE NEW ZEALAND JOURNAL OF

medical laboratory technology

An Official Publication of the New Zealand Institute of Medical Laboratory Technology Incorporated

VOLUME 33, No. 2

PRODUCT and PRICE!

MERCK gives you both.

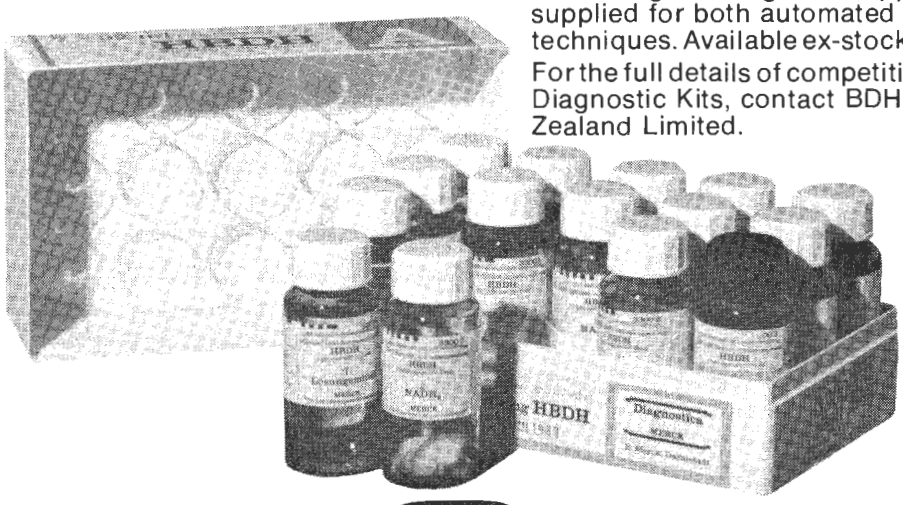
Deep in the heart of the Black Forest when traditional cuckoo clocks were first hand crafted, the product was admired by all, but afforded by few.

Likewise, Merck of Darmstadt (West Germany) have always manufactured superior products much admired in their field . . . products which in the earlier days carried a premium price.

Traditional cuckoo clocks are no longer individually crafted, but still as sought after. Just like the Merck range of products, they are marketed universally at a much lesser price.

A full range of diagnostic applications can be supplied for both automated and manual techniques. Available ex-stock Palmerston North.

For the full details of competitively priced MERCK Diagnostic Kits, contact BDH Chemicals New Zealand Limited.



BDH

BDH Chemicals New Zealand Ltd
Tremaine Avenue, PO Box 1246, Palmerston North.
Telephone 82-038, Telex 31476.

The New Zealand Journal of Medical Laboratory Technology

Volume 33, No. 2 July, 1979

The JOURNAL is published three times yearly (in March, July and November), and is distributed, without charge, to all financial members of the N.Z.I.M.L.T. (Inc.).

Subscription to the JOURNAL for non-members is SIX DOLLARS per year or TWO DOLLARS FIFTY CENTS per single issue, postage paid. Overseas subscription rates on application.

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

Inquiries regarding advertising rates and copy or blocks for advertising should be addressed to the Advertising Manager, Allied Press, P.O. Box 181, Dunedin.

Communications primarily concerning the Institute should be addressed to the Secretary.

Changes of address should be notified promptly to the Convener of the Membership Sub-committee, Mr C. S. Curtis, P.O. Box 52, Hamilton.

Betatest®

The test for Organisms that are Penicillin Resistant due to B-Lactamase production

- INSTANT IDENTIFICATION OF RESISTANT ORGANISMS
- IMMEDIATE RESULTS FROM PRIMARY CULTURE
- SPEEDS CORRECT CHEMOTHERAPY
- SIMPLE FOR ROUTINE SURVEILLANCE
- EASY TO SCREEN NEW ISOLATES:

Campylobacter	Gonococcus
Haemophilus	Meningococcus
Bacteroides	Salmonellae
Fusobacterium	E. Coli

Shigella

- IDEAL FOR SCREENING GRAM NEGATIVE ISOLATES FROM PATIENTS RECEIVING PENICILLIN & ALLIED ANTIBIOTICS

●●●ALL ADDS UP TO OBVIOUS PATIENT BENEFIT●●●

Manufactured & Supplied by

Medical Wire & Equipment Co(Bath)Ltd

Write for leaflet to N.Z. Agent:
CARTER CHEMICALS LTD, P.O. BOX 1326, DUNEDIN, TELEPHONE 779-359

Evaluation and Comparison of Serum Phenytoin Assay by Gas Chromatography and Two Enzyme Multiplied Immunoassay Techniques

Christine Chapman, QTO[1], Diana Kappatos, BSc[2], R. W. L. Siebers, ANZIMLT

Division of Chemical Pathology, Department of Laboratory Services, Wellington Hospital

Received for publication September 1978

Summary

Phenytoin was measured in patients' sera by three analytical procedures: gas liquid chromatography; enzyme immunoassay technique using a semi-automated method and the enzyme immunoassay technique adapted to the Abbott ABA 100.

The results of the three procedures showed good precision and reliability. Between batch coefficients of variation, determined by repeated analysis of a 60 μmol/l control sample, were: GLC: 3.1 percent; semi-automated EMIT: 4.3 percent; and automated EMIT: 4.3 percent. The correlation coefficients were: GLC v semi-automated EMIT 0.952; GLC v automated EMIT 0.966; and Semi-automated EMIT v automated EMIT 0.963.

In nine patients out of the 110 studied the EMIT procedures consistently gave results that were significantly different from results obtained by GLC. Our results show that the three methods are generally interchangeable; however users of EMIT should be aware of the presence of interfering substances in the sera of some patients.

Introduction

Phenytoin is the most widely used anticonvulsant drug and monitoring of its concentration in serum is well documented. GLC is the usual method of analysis but the enzyme multiplied immunoassay technique (EMIT) now offers an attractive alternative. In this study phenytoin levels were assayed by three procedures: GLC; a semi-automated EMIT; and an automated EMIT adapted to the Abbott ABA 100. The three methods were compared in terms of precision and accuracy.

Materials and Methods

Serum was obtained from blood samples submitted to the laboratory for routine anticonvulsant drug determinations. The sera were stored at —40°C between analyses.

Gas Liquid Chromatography

A Varian 3700 Gas Liquid Chromatograph equipped with dual hydrogen flame detectors was used. A glass column 2m long and 3mm id was packed with 3 percent OV 17 on 80/100 mesh Chromosorb WNAH (Superelco).

Carrier gas flow was 30ml/min, hydrogen 34ml/min and air 340ml/min. Column temperature 250°C, injector port temperature 360°C and detectors 300°C.

Extraction and methylation of phenytoin were performed as described by McGee (1970)[4]. A calibration curve was prepared each day by adding phenytoin in methanol to pooled drug-free serum. Peak heights were used for quantitation.

EMIT Semi-automated Procedure

The procedure was carried out according to the package insert supplied with the EMIT Assay Kit for phenytoin (Syva Corp). A Beckman model 24 Spectrophotometer, equipped with a sipper and thermally regulated flow cell, was used to measure enzyme activity. A Syva printer/calculator was integrally connected to the spectrophotometer. All serum and reagent dilutions were made with a Syva pipetter/diluter.

A standard curve was made each day using the EMIT Calibrator set. All patient samples were analysed in duplicate.

1. Present address: Chemistry Department, Victoria University, Wellington.
2. Present address: Wellington Clinical School.

EMIT Adapted to the Abbott ABA 100

The EMIT antibody reagent A, enzyme reagent B, AED buffer and calibrators were reconstituted according to the package insert supplied with the EMIT Assay Kit for phenytoin. A 1:30 dilution of the enzyme reagent B was made with the AED buffer. Instrument parameters were as follows:

Incubator	30°C
Mode selector	Rate
Reaction direction	Up
FRR/Normal switch	Normal
Analysis time	5 minutes
Carousel revolution	3
Filter	340/380
Syringe plate	1:101
Sample size	5 μl
Auxiliary dispenser	10% stop to dispense 25 μl
Calibration factor	0.500

Calibrators and samples were placed in the sample cups beginning with position 2. Distilled water was placed in position 1. Reagent lines were attached to the dual probe and the probe adjusted to deliver the reagents down the side wall of the cuvette. Zero and calibration factors were set and the run started. A standard graph was established each day with the EMIT calibrator set. All patient samples were analysed in duplicate.

Quality Control

Two control samples used for all methods were prepared by adding phenytoin in methanol to pooled drug-free serum to a concentration of 20 μmol/l and 60 μmol/l. The control samples were stored in aliquots of 10ml at —40°C until used.

Statistical Analysis

Statistical analyses were performed using a PDP 11/40 computer.

Results

Within batch precision for the GLC and automated EMIT methods was determined by repeated analysis of two samples. The results are presented in Table 1 and show good precision for each method.

Between batch precision for all three methods was determined by analysing two sera spiked with phenytoin at a concentration of 20 μmol/l and 60 μmol/l. The results are presented in Table 2 and show good precision for each method. The coefficients of variation for the 2 EMIT methods are better than the 15 percent allowed for by the manufacturers.

The statistical analysis of the correlation between the three methods is presented in Table 3. The results obtained for the correlation coefficient, slope and intercept show close agreement between the three methods.

Table 1.—Within-Batch Precision Studies of the GLC and Automated Abbott ABA 100 Methods.

	n	x	S.D.	C.V.%
		μmol/l		
G.L.C.	32	48.4	1.37	2.83
	32	84.0	1.49	1.77
E.M.I.T.	44	19.6	0.69	3.50
(ABA 100)	44	52.5	1.20	2.30

Table 2.—Between-batch Precision of the G.L.C. and E.M.I.T. Methods

	n	x	S.D.	C.V.%
		μmol/l		
G.L.C.	42	20.4	1.33	6.5
	48	60.5	1.88	3.1
E.M.I.T.	61	22.0	1.84	8.4
(Semi-automated)	87	60.3	2.60	4.3
E.M.I.T.	18	21.2	1.96	9.2
(ABA 100)	21	60.0	2.60	4.3

Table 3.—Statistical Comparison of GLC, Semi-automated EMIT and Automated EMIT

	m	b	a	r	n
		μmol/l			
S.A. E.M.I.T. vs G.L.C.	0.986	—0.12	+1.17	0.952	98
S.A. E.M.I.T. vs A. E.M.I.T.	0.948	+0.80	+1.17	0.963	95
A. E.M.I.T. vs G.L.C.	1.041	—0.72	—0.73	0.966	95

S-A E.M.I.T. = Semi-automated E.M.I.T.
A.E.M.I.T. = Automated E.M.I.T.
m = Slope
b = Intercept
a = Average mean difference
r = Correlation coefficient
n = number of cases.

. . . the new name in your laboratory

Sclavo Diagnostics introduces a comprehensive range of kitsets for the Clinical Biochemistry Laboratory.

Currently available:

Enzymatic Chemistries

CK-UV, AST-UV, ALT-UV, LDH-UV, HBDH-UV, ∝-Amylase, Alkaline Phosphatase, Glucose (GOD-POD) and Cholesterol (CHOD-POD)

General Chemistries

Albumin, Bilirubin, B.U.N., Creatinine, Haemoglobin, Total Protein and Uric Acid

Standards

Albumin, Bilirubin, Haemoglobin

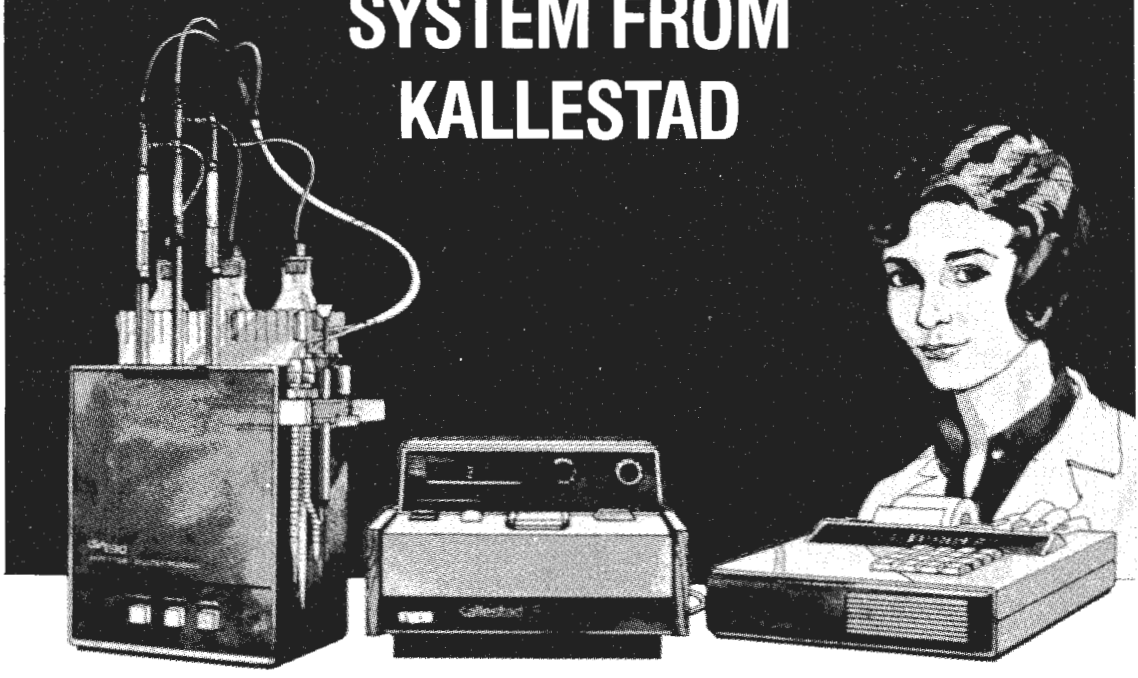
Application sheets are available for continuous flow and discrete analysis, including:

- Abbott ABA 100
- Centrifichem
- Gilford 3500
- AA-I, AA-II, SMA 6/60 and SMA 12/60

Sclavo products are now available from:

Laboratory Services Limited
PO Box 12-502,
Penrose, Auckland 6

NEW
THE LSA 290™ NEPHELOMETRIC SYSTEM FROM KALLESTAD



PIPETTER/DILUTER MODULE

- Dilutes patient sample and pipettes assay reagents in one precise step.
- Significantly reduces technologist time.
- Applicable to other laboratory procedures.
- Compact and easy to operate.

LSA 290 NEPHELOMETER

- Light scattering technology substantially reduces assay time.
- Precise and reproducible results.
- Highly sensitive, easy to operate.
- No constant monitoring required.
- Compact and portable.

COMPUTERIZED DATA PROCESSING MODULE

- Computerized Data Reduction Module which interfaces with the LSA 290 Nephelometer — eliminates time consuming data handling.
- Digital Display and printer for permanent record.
- Memory capabilities for storing standard curve data.

SMITH BIOLAB

SCIENTIFIC DIVISION

P.O. Box 36007,
Auckland 9, New Zealand

Auckland	Ph. 483-039
Wellington	Ph. 683-453
Christchurch	Ph. 63-661

WITH RESIDENT REPRESENTATIVES IN HAMILTON, PALMERSTON NORTH AND DUNEDIN.

150404

Discussion

There is a good correlation between the results obtained by the GLC, semi-automated EMIT and automated EMIT methods. Student's t test shows no significant difference between the methods at the 95 percent confidence limits and the F test shows no significant difference between the precision of the three methods. Our results generally agree with the findings of other studies comparing GLC and EMIT.[1,2,3,5]

Despite the good correlation between the three methods there were nine patients out of 110 whose results were consistently significantly different, that is greater than two standard deviations from the mean. When the EMIT procedure was originally introduced in our laboratory, an entirely manual technique was used. The aberrant results obtained for these nine patients were thought to be due to faulty technique, however, similar results were later obtained with both the semi-automated method recommended by Syva Corp and the automated Abbott ABA 100 technique. Serial samples were analysed in three of these nine patients and the results of one, patient H, are presented in Table 4. The results of the GLC and EMIT analyses are significantly different on four of the five days that blood was collected. Our GLC results were confirmed by a double blind analysis using a different GLC method in another laboratory.

Patient H was an inpatient and was on regular treatment with phenytoin, digoxin and pericyazine throughout the study period. The patient had a history of ischaemic heart disease, was in chronic renal failure and had developed hyperchloraemic acidosis by 27 June 1977. Serial samples analysed in the other two patients also showed significantly different results (unpublished). In two of the three patients the dose of phenytoin was reduced during the time the serial samples were taken. It was found that as the serum phenytoin levels decreased the results obtained by EMIT and GLC fell into agreement. The only drug administered which was common to all nine patients was phenytoin. Patient H was the only patient in renal failure and there was no other apparent clinical condition common to all patients.

Table 4.—Phenytoin Analysis in Patient H.

Date	E.M.I.T. (semi-auto)	E.M.I.T. (ABA 100)	G.L.C.	G.L.C.	Daily Dose
	μmol/l				mg
17/5/77	104	63	47	48	300
25/5/77	106	119	52	56	300
10/6/77	91	63	42	42	300
27/6/77	27	26	22	—	200
8/7/77	72	44	29	30	300

Therapeutic Range 40-80 μmol/l.
G.L.C. results Wellington Hospital Toxicology Unit.
G.L.C. results Christchurch Hospital Toxicology Unit.

Due to the good correlation between the two GLC methods and the fact that we consistently obtained aberrant results in these nine patients when using EMIT techniques, we assume that there was interference with the EMIT procedure. As the aberrant results were present in the same patients using three different EMIT techniques, they would appear not to be due to random technical errors. Results reported by Spiehler *et al.* (1976)[5] also suggested interference with phenytoin assay in sera of some patients when using the EMIT procedure.

All three procedures evaluated were precise and accurate. The EMIT procedure was more rapid and used a smaller sample volume than did GLC analysis. The automated Abbott ABA 100 technique processed the samples about three times more rapidly than the semi-automated technique, allowing the analysis of approximately 400 samples in a working day.

Acknowledgments

The authors wish to thank Syva Corporation, Palo Alto, California for the use of the automatic pipetter/diluter and timer printer and the supply of some of the Kits used in this study; Abbott Australia Ltd, for the loan of the ABA 100; Christchurch Hospital Toxicology Unit for confirmation of GLC results; Dr's H. Ford and B. Casey and Mr W. Chisnall for their valuable comments; and Ms Y. Lee for technical assistance.

REFERENCES

‡. Bastiani, R. *Clinical Study No. 20, Summary Report, Syva Co.*

2. Brokker, H.E., and Darcey, B.A. (1975), *Clin. Chem.*, **21.**
3. Liqaz, M., and Raisys, V. (1976), *Clin. Biochem.*, **9,** 35.
4. McGee, J. (1970), *Anal. Chem.*, **42,** 421.
5. Spiehler, V., Sun, L., Miyada, D.S., Sarandis, S., Walwick, E.R., Klein, M.W., Gordon, D.B., and Jessen, B. (1976), *Clin. Chem.*, **22,** 749.

Preliminary Surveys of Instrument Calibration in New Zealand Clinical Laboratories

M. Lever, D. J. Munster and F. B. Desmond

Clinical Biochemistry Department, Christchurch Hospital, and Medical Laboratory, Wellington.

Received for publication, August 1978

Summary

New Zealand clinical laboratories were sent unknown calibration materials for balances (weights), spectrophotometers (acid dichromate solution) and pH meters (buffers at pH 4.3 and 9.1). The frequency of balance miscalibration suggests that this probably has a negligible effect on interlaboratory variability. Variations in spectrophotometer performance may contribute significantly to interlaboratory variation in some methods, and in at least some enzyme assays it is likely that pH meter calibration is a major factor in causing large differences between laboratories. Although an interlaboratory survey of instrument calibration can play only a small role in the external audit of internal control procedures, such a survey provides useful information about possible sources of error, and about areas of laboratory practice which require improvement.

Introduction

Surveys in which instrument calibration materials are distributed to laboratories, and the reported results compared, are not new. Spectrophotometric standard solutions have been distributed several times by organisations such as the College of American Pathologists (CAP)[1,3] and the Royal College of Pathologists of Australia[4]; balances have also been studied by the CAP[2].

As part of a research programme into the design of interlaboratory surveys, three calibration surveys of New Zealand laboratories were carried out, dealing with balances, spectrophotometers, and pH meters. These were intended to develop practical procedures for New Zealand conditions, and to investigate the role of such surveys in any overall programme for the external audit of the ability of laboratories to meet clinical requirements.

Methods

Balances

Thin stainless steel sheet was cut into approximately rectangular pieces of varying sizes. Each piece had a corner turned up and an identification number stamped on it; these stainless steel pieces are referred to as the weights in this paper.

Each weight was weighed on two occasions by different operators using a Mettler H33 balance which had been calibrated by a TELARC registered service company, and which was supported on a concrete beam in a modern building. Five of the weights were then sent to the Physics and Engineering Laboratory (PEL) of the Department of Scientific and Industrial Research (DSIR) in order to check the calibration of the balance. Each participating laboratory was then sent a pair of weights, one 'high' (260 to 1370 mg) and one 'low' (30 to 460 mg), and asked to return their estimates of the weights.

Spectrophotometers

A solution of acid dichromate was used (50 mg $K_2Cr_2O_7$ dissolved in 1 litre 0.005 M

H_2SO_4 for surveying spectrophotometer calibration at 340 nm. Each participating laboratory was sent two sealed glass vials; one containing dichromate solution and one containing a blank (no dichromate). Each laboratory was asked to measure the absorbance of the dichromate solution at 340 nm, or other wavelengths used for NAD(P)H quantitation. Samples were also sent to the Chemistry Division of the DSIR, Petone, for accurate calibration at 340 and 350 nm using their Beckman Acta MIV spectrophotometer. The DSIR also provided scans of the absorbance spectrum of the dichromate solution between 300 nm and 385 nm. The absorbance scale was calibrated against a 50.0 mg/l solution of potassium dichromate (Riedel de Haen AG) in 0.010 N H_2SO_4, using triple-distilled water (last distillation from alkaline permanganate) (personal communication). Laboratories should note that dichromate solutions will have lower absorbances than theoretical unless stringent precautions are taken in their preparation, and that we do not recommend the use of dichromate solutions as primary calibration standards.

pH Meters

Two buffer solutions were supplied by the Chemistry Division of the DSIR, Petone (Appendix I). A specimen of each of these was distributed in a rubber-stopped glass specimen tube (Venoject, plain) to each participating laboratory, and the laboratories asked to measure the pH with the instruments which they normally use in reagent preparation.

Results

Balances

The calibration data on the five weights sent to the DSIR (Table 1) show that the assigned values for the weights are close to the reference values; there is possibly a slight underestimation (less than 0.5 mg). In all 118 weights were weighed by the two operators, and in only 12 cases were discrepancies greater than 0.5 mg obtained; the largest was 1.0 mg (in 530 mg). Some weights were also weighed on another analytical balance by a third operator.

Four laboratories made obvious reading or transcription errors in their returns; these were noted, then corrected before further analysis of the data. The errors were: two laboratories reported results in error by a factor of 10; one laboratory reported 1994.9 mg instead of 994.9 mg; and the other case was a transposition of high and low results.

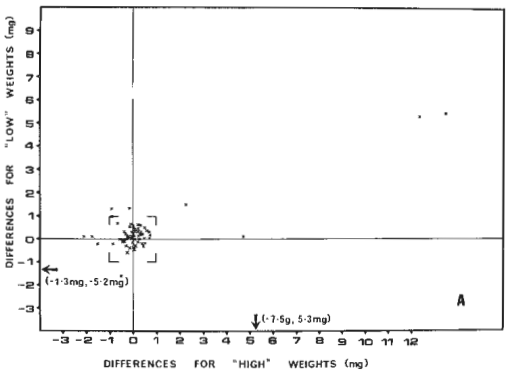


Figure 1.—Youden diagram presentations of differences between weight calibration results and reported results in mass units (mg). Square brackets enclose the 1 mg difference, which cannot be attributed with certainty to miscalibration or misreading.

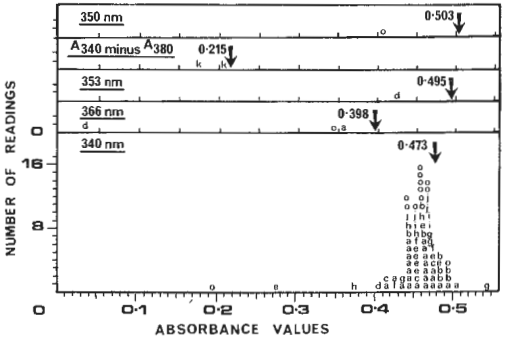


Figure 2.—Absorbance values reported by laboratories for dichromate solution. Arrow indicates target value (10 mm light path). Instrument categories given in Table II.

The great majority of laboratories reported results which should be sufficiently accurate for preparing standards for clinical chemical analysis (Fig. 1). Only 18 of 118 weighings were in error by more than 1.0 mg; or, in terms of percentages, only 12 weighings were in error by more than 1 percent. Some laboratories weighed the weights on several balances, including some not designed for high accuracy and probably not used for weighing

Table 1.—Calibration of weights by DSIR

Code no.	DSIR	Weight (mg) Operator 1	Operator 2	Estimates Our Value
150	1,243.89	1,243.8	1,244.0	1,243.9
130	710.55	709.7	710.4	710.1
83	361.43	361.5	361.1	361.3
103	188.95	188.7	189.1	188.9
26	36.47	36.2	35.9	36.2

standards. Five laboratories were in error by more than 1 mg for both weights, suggesting the possibility of incorrect balance calibration in these cases. The laboratories (Fig. 1) with the largest errors were written to individually, and in one case a reply was received acknowledging that an error had been made in setting the zero point, and stating that this error was not likely to be repeated in routine weighings.

Spectrophotometers

Calibration data for the spectrophotometer standard solution are given in Appendix I. Eighty-two results were returned from 50 laboratories. These results were mostly between absorbances of 0.4 and 0.5 at 340 nm (Fig 2), compared with a target value of 0.473. Since the absorbance maximum of the acid dichromate solution is at 350 nm (calibration absorbance 0.503), the high values returned by some laboratories could have been caused by errors in wavelength calibration; however, the predominance of low results suggests that other problems such as stray light could be common.

The data in Figure 2 suggest that differences between types of instrument play at most a small part in the range of reported absorbances. There is possibly a tendency for the Calbiometer to read a little high. Instruments used are given in Table 2, which also explains the codes used in Figure 2.

Overall, the interlaboratory variation in absorbance readings appears to be smaller than the interlaboratory variation in test results which are calculated from absorbance readings.

pH Meters

One obvious reporting mistake (high pH and low pH results transposed) was amended without comment. After this, the pH values

Table II.—Instruments used for absorbance measurements

Code in Fig. 2.		Number included
a	Gilford 300 series	25
b	Calbiometer LC 340	8
c	Bausch & Lomb Spectronic 100	3
d	Pye Unicam SP 600	3
e	Pye Unicam SP 800, 1700, 1800, 8000	8
f	Pye Unicam SP 30	3
g	Beckman DB	5
h	Beckman 24, 25	4
i	Shimadzu QV50	3
j	Varian Techtron 635	4
k	Abbott ABA	2
o	Only one of its type, or insufficient information	15

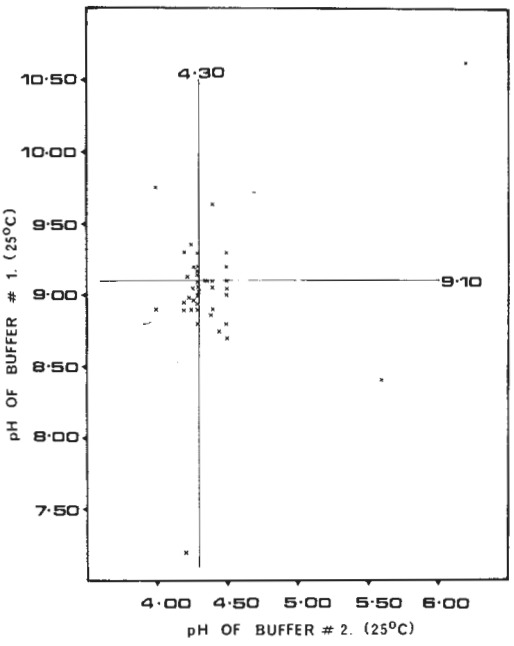


Figure 3.—pH values reported by laboratories corrected to 25°C and presented on a Youden diagram.

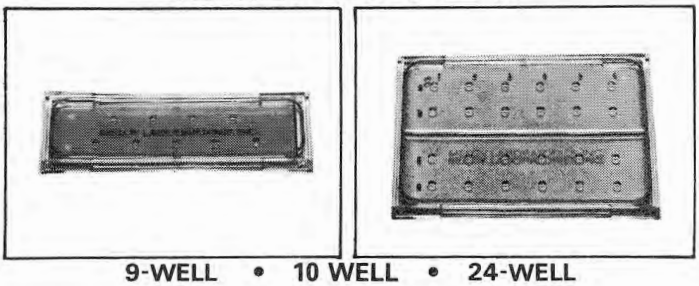
MELOY....

Most Complete Line Of Immunochemical Products

AVAILABLE FOR IMMEDIATE DELIVERY

NEW
EACH IMMUNODIFFUSION KIT INCLUDES STATISTICAL DESCRIPTION OF THE REFERENCE STANDARD CURVE

IMMUNODIFFUSION PLATES



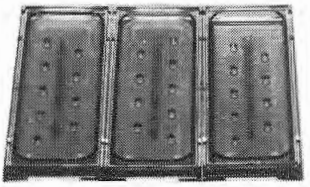
9-WELL • 10 WELL • 24-WELL

Also available in packages of plates only.

IgG, IgM, IgA, IgD
Albumin
Alpha$_1$ Antitrypsin
Ceruloplasmin
Alpha$_2$ Macroglobulin
Transferrin
Hemopexin
C'3, C'4, C'5
IgE
Low Level IgG, IgA, IgM
Bence-Jones Kappa & Lambda
Polyvalent Immunoglobulins
Whole Human Serum
ANA Kit (for LE)
AMA Kit
T3, T4, T3 Uptake, Neonatal T4 RIA

NEW
Endpoint Kits
1gA, M, G, C3, C4
Anti-Trypsin

ANTISERA



AGM PLATE

ALSO AVAILABLE – Fluorescein Conjugated Antisera (human and animal), Mouse Immunoglobulin Plates, Abnormal Reference Sera, Radioimmunoassay

MELOY

The Immunology People

Sole N.Z. Agents

GENERAL DIAGNOSTICS [NEW ZEALAND] LTD.

206 THE PARADE, ISLAND BAY, WELLINGTON, 2. P.O.BOX 7170 WELLINGTON SOUTH
CABLES & TELEGRAMS: DIAGNOSTIC. TELEX: GENDIAG NZ 3859. TELEPHONE 836-255

1204

reported were corrected to 25°C. This correction obviously caused some confusion with participants; the confusion is between temperature compensators (often automatic) on the instruments, and pH variations with temperature in the buffer solution. The former correct for electrode response, whereas the latter are caused by changes in the pK of the weak acid used in each particular buffer. The corrections we made were for the latter.

Results were returned from 45 laboratories. Some laboratories reported readings obtained from different instruments, and in one case a large discrepancy between results from two instruments in the same laboratory was reported without comment.

The results (Fig 3) show that pH meters are often poorly calibrated, especially at high pH levels. A possible reason for this is that relatively stable (phthalate) standard buffers are used to calibrate instruments at neutral and low pH levels, and checks at high pH levels are less commonly made. Most common malfunctions in pH meters are readily detected by calibration with two buffers of different pH. To ensure reliability, dual calibration is essential.

Discussion

Interlaboratory surveys can have a number of functions. It has been argued elsewhere[6] that the main function is to audit externally the ability of the laboratory to maintain the analytical standard required for adequate patient diagnosis and management. However, there are other possible functions; laboratories can be helped to diagnose specific problems by information concerning the forms of error present, and reasonably objective data can be obtained about the analytical performance of participating laboratories. For each of these possible functions, a survey of basic laboratory calibration could arguably replace part or all of a conventional 'round-robin' survey using actual analytical samples. Our results to date would not support this view, but even if it is found that such a survey cannot replace the round-robin, it may be a useful adjunct.

Audit Function

The three calibration techniques studied in this paper were chosen as those most likely to contribute to variations in laboratory performance, temperature being the main omission. However, many instruments, such as continuous flow colorimeters, are not easily checked, and these are used in the production of many test results. It is unlikely that the observed interlaboratory variation in instrument calibrations could completely account for the variations in reported test results, and few would assume that calibration surveying alone could audit the internal quality control of laboratories. However, regular maintenance and recalibration of instruments is an essential part of good laboratory practice, and an external audit of this may be regarded as part of the function of a comprehensive survey.

The overall performance in the calibration survey also informs professional societies and those responsible for educating laboratory staff where there are some deficiencies in maintenace, and provides objective data on the magnitude of these.

Results of Other Calibration Surveys

Some comparisons can be made between the results obtained in this study and those obtained in other surveys. The CAP study of balance calibration[2] also found that this appeared to be a negligible source of interlaboratory variability. The present study of spectrophotometer calibrations shows a moderate degree of variability similar in magnitude to CAP studies [1,3] and much smaller than in the Australian study[4]. The Australian results may, in part, be an artefact of some relatively unstable calibration solutions, since it is hardly likely that New Zealand laboratories perform markedly better than their counterparts in Australia.

Other Survey Functions

The errors in spectrophotometer calibration could make a substantial contribution to interlaboratory variability in the many methods which involve measuring changes in absorbance at 340 nm (interconversion between NAD (P)$^+$ and NAD(P)H: those laboratories which reported erroneous results in the spectrophotometer survey could be led directly to identifying a source of error in such methods). From the errors found in pH meter calibration at pH 9.1, and from known differences bet-

ween isoenzymes of human and animal origins[5], one could predict a large interlaboratory variation in alkaline phosphatase estimates.

It is also useful information to know that primary balance calibration contributes very little to interlaboratory variability.

Acknowledgments

Financial support was provided by the Medical Research Council and participating laboratories. We appreciate the co-operation of laboratory staff in all parts of New Zealand, and thank the scientific staff of the DSIR who provided basic calibration data and materials. Numerous discussions with our colleagues, both in the hospital service and the DSIR, have contributed to this work: their importance cannot be adequately covered in this brief note. We particularly wish to thank Dr R. B. Williamson for absorbance calibrations, Dr C. D. Stevenson for the buffer solutions, and Dr M. Kingsford for advice and discussions.

Some repetitive passages deleted, Editor.

REFERENCES

1. Beeler, M. F. (1973), *Amer. J. Clin. Pathol.* **61,** 789.
2. Beeler, M.F. and Boudreau, D.A. (1977), *Amer. J Clin. Pathol.* **68,** 207.
3. Beeler, M. F. and Lancaster, R. G. (1975), *Amer. J. Clin. Pathol.* **63,** 953.
4. Chemical Pathology Committee (1977), *Report of Quality Assurance Programmes for 1975-77.* Royal College of Pathologists of Australia Board of Education, Melbourne.
5. Fowler, R. T. and Walmsley, T. A. (1978), *Clin. Chim. Acta* **87,** 159.
6. Lever, M. and Munster, D. J. (1978), *N.Z. J Med Lab. Technol.*, in press.

Two Unrelated Cases of Factor XII Deficiency

G. S. Milicich ANZIMLT

Coagulation Laboratory, Haematology Department, Wellington Hospital

Received for publication, January 1979

Summary

Several hundred cases of Factor XII deficiency or Hageman Trait have been reported since Ratnoff and Colopy in 1955 described the first case. With few exceptions these patients have no history of abnormal bleeding. This paper reports two unrelated cases of severe Factor XII deficiency diagnosed within three months of each other.

Introduction

Factor XII is involved in many pathways, necessary in blood coagulation; Kinin generation, intrinsic activation of plasminogen to plasmin and in activation of the complement system. Recent reviews of Factor XII deficiency report increased incidences of cerebro-vascular accidents, thrombo-embolic, disease, easy bruising, attacks of local oedema, allergic reactions, and both severe headaches and abdominal pain.

Case Histories

Case A—40-year-old female.

On examination at Wellington Hospital the patient had a history of nose bleeds at age 20, bruised easily and had always had heavy periods. She was to have a hysterectomy because of this. The only suspicious family history was from her father's sister who suffered with easy bruising and possible bleeding problems.

Case B—45-year-old male

This man was admitted to Wellington Hospital for Cardiac Catherisation and mentioned to the Doctor that his family in Australia had been diagnosed as Factor XII deficiency. He presented with no clinical history of any bleeding problems.

Investigations

In Case A we were able to perform full family studies and other investigations

that we are now the sole N.Z. agents for

Medical Wire & Equipment Co (Bath) Ltd England

WHO OFFER THE MOST COMPREHENSIVE RANGE OF SWABS

for all enquiries and service
contact the exclusive N.Z. Agents
Carter Chemicals Limited
Telephone 779-359
P.O. Box 1326, Dunedin.

CARTER CHEMICALS LIMITED

Qualicheck®.

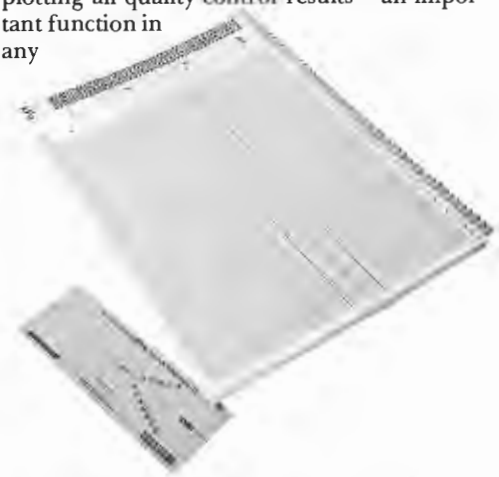
A quality control program developed by the worlds most experienced producer of blood gas analyzers.

Dispenser

The Radiometer Qualicheck® System reflects our experience as a leader in the blood gas field. The unique dispenser, for example, assures anaerobic transfer of the quality control liquid to the analyzer.

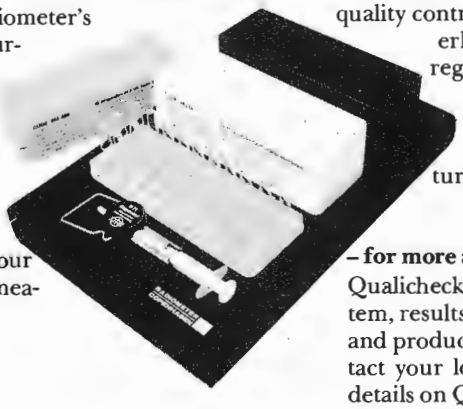
Data Collection

The Qualicheck® logbook is convenient for plotting all quality control results – an important function in any quality control program. All pages – again properly color coded – will, when used on a regular basis, allow for a safe and quick evaluation of an analyzer's reliability. The temperature templet reduces errors due to room temperature variations and eliminates the need for a constant temperature bath.



Control Liquids

Qualicheck® includes three different check solutions covering the clinically significant levels. The bright color coding of all material eliminates potential confusion. Each batch of Qualicheck® is carefully analyzed so that assigned values for each level become an integral part of Radiometer's total quality assurance program. The solutions are sealed in single-use ampoules, providing the most favorable conditions for your quality control measurements.



– for more about Qualicheck®

Qualicheck®, Radiometer's quality control system, results from our experience in developing and producing blood gas instrumentation. Contact your local Radiometer representative for details on Qualicheck® and quality control.

RADIOMETER COPENHAGEN

Radiometer A/S · 72 Emdrupvej
DK-2400 Copenhagen NV · Denmark

Sole New Zealand Agent:

WATSON VICTOR LIMITED
P.O. Box 1180, Wellington

Also at:

Auckland Christchurch Dunedin

J+J Industrial advertising/Copenhagen

described below.

In Case B the only work performed at this hospital was on the patient while in hospital.

As the original patient Mr Hageman died of a massive pulmonary embolism and because of the reported increased incidence of thromboembolic disease, we decided to look at this aspect by performing Antithrombin III assays.

One case of Factor XII deficiency has been reported which was associated with abnormal chromosome pattern, so chromosome studies were performed in Case A on selected members of her family.

Platelet adhesion is said to be abnormal in Factor XII deficiency—this was only measured in Case A on the patient's father.

Partial Thromboplastin Time (PTT) was performed using Pathromtin from Behring Institute.

Factor XIII, IX, XI and XII assay were performed using Dade substrate plasmas and Actin.

Antithrombin III assays were performed using a modification of the method of Dacie *et al.* (1975)[3].

Platelet Adhesion method of Rae (1974)[8].

When the two plasmas of Case A and Case B were mixed together no correction was obtained in the PTT, suggesting that both patients have the same defect.

Discussion

Factor XII deficiency is usually inherited as an autosomal recessive trait and affects the sexes equally. It is thought that there are at least two allelomorphic genes that control the synthesis of Factor XII.

H^n-associated with higher levels of Factor VII

H^1-associated with lower levels

Two abnormal alleles were also suggested

H^0-for absence of Factor XII

H^m-for moderate deficiency.

In Case A we can postulate that the patient's father carried the abnormal allele H_0 (perhaps he is H^1/H^0) and that in this family the expression H^1/H^0 gives rise to no Factor XII production thus explaining the Factor XII level of < 1 percent in the two sisters.

This family did not appear to suffer from any particular clinical problem, although the patient's brother did suffer from Hay fever (11 percent Eosinophils when tested) and her father had had a skin complaint for many years. Are these facts related to the other functions of Factor XII?

On reviewing previous reports of Factor XII deficiency, menorrhagia in affected females seems to be a reasonably consistent finding.

In Case B no history of any particular clinical problem has been found in any members of his family.

A question which must be answered, is why does Factor XII deficiency generally not cause

Results

Case A

	PTT (N 35-45 secs.)	Factor XII assay (N >55%)
Patient	>360 sec	<1%
Family—sister (1)	345 sec	<1%
sister (2)	42 sec	70%
mother	38 sec	66%
father	58 sec	50%
	PTT	XII
Sibs. daughter (1)	57 sec	38%
daughter (2)	49 sec	56%
son	53 sec	48%

Other significant results were:

Lee and White clotting time on patient 20 mins.

Factor XI, VIII and IX assays were normal on patients and all her children.

No inhibitors were detected on affected members.

Antithrombin III assays on affected sisters were both normal.

Chromosome studies on patient, her mother, father and affected sister were all normal.

Platelet adhesion was normal on patient's father.

Case B

		Factor XII Assay (N 55)
Patient	< 240 sec	>1%
Lee & White clotthing time 24 mins		
Family (tested in Australia)	brother (1)	<1%
	brother (2)	100%
	brother (3)	100%
	sister (1)	<1%
	sister (2)	36%
	sister (3)	36%
Children of brother (1)	daughter	20%
	son (1)	40%
	son (2)	40%
	son (3)	40%

a bleeding disorder? There are three theories to explain this:

1. Hageman factor deficiency although absent from plasma is present in tissues and is released at a site of injury.
2. There is an alternative coagulation pathway bypassing Hageman factor.
3. Absence of Hageman Factor not only results in defective coagulation, but also results in alteration in other physiological mechanisms which compensate for Hageman Factor deficiency.

Conclusion

This paper shows two cases of Factor XII deficiency of doubtful clinical significance.

We would expect to find more members of the family in Case A to have reduced Factor XII levels.

It also raises the point that more rare coagulation deficiencies may be unearthed in this country with the increased incidence of blood testing.

One wonders how many more Factor XII deficient people remain undetected?

Acknowledgment

I wish to thank Dr G. Green and Mr E. Crutch of Wellington in encouraging me to present this paper, Mr D. Fisher of Masterton Hospital for bringing the patient in Case A to our attention and Ms D. Scott of Brisbane for the information in Case B.

REFERENCES

1. Bigg, R., (1975), *Human Blood Coagulation,* Blackwell Scientific Publications.
2. Colman, R. W. and Wong, P. Y. (1977), *Thrombosis Haemostas.* **38,** 751.
3. Dacie, J. V. and Lewis, S. M., (1975), *Practical Haematology.* 365.
4. De Groughy, J., Veslot, J., Bonnette, J. and Roidot, M., (1968), *Am J Dis Child.,* **115,** 93.
5. Egeberg, O., (1970), *Thrombosis & Diathesis Haemorrhagica.* **23,** 432, 441.
6. Girolami, A., Faffei, A., Brunetti, A. and Lazzarin, M., (1970), *Thrombosis & Diathesis Haemorrhagica.* **23,** 449.
7. Poller, L., (1977), *Recent Advances in Blood Coagulation.* 49 & 68.
8. Rae, B. A., (1974), *NZ J Med Lab Technol* **28.** No. 3. 83.
9. *Thrombos Haemostas* (1978), Letters to the Editor **39.** 234.

A Comparison of the Modified Rapid Fermentation Test and Fluorescent Antibody Test for the Identification of Neisseria Gonorrhoeae

Shirley Gainsford

Microbiology Division, Department of Laboratory Services, Wellington Hospital

Received for publication, October 1978

Introduction

We have found the Fluorescent Antibody (FA) test to be a quick and specific test for identifying *Neisseria gonorrhoeae.* However it is necessary to have a confirmatory test when fluorescence is doubtful. Such a test would rely on carbohydrate fermentation, and cysteine trypticase agar sugars have been used. These require 24-48 hours of incubation and as the Modified Rapid Fermentation Test (MRFT) has been shown to give results in 2-4 hours[1], we decided to investigate this test in parallel with the FA test.

Materials and Methods

One hundred oxidase positive gram negative diplococci from patients attending the Venereal Disease Clinic, Wellington Hospital over a three-month period were isolated on Thayer Martin (TM) medium and subcultured onto GC Agar base enriched with haemoglobin and isovitalex but without antibiotics.

Fluorescent Antibody Test

A suspect colony from the primary TM plate was suspended in a drop of distilled water on a glass slide and allowed to air dry. The smear was then covered with a drop of a

working dilution of Bacto F.A. *N. gonorrhoeae* conjugate and incubated at room temperature for five minutes then washed in distilled water.

The dried slide was mounted in glycerol saline and examined under a 100X objective using a Leitz microscope with a BG12 exciter filter and K530 barrier filter. Positive and negative controls of *N. gonorrhoeae* and *Neisseria species* were included in each batch.

Modified Rapid Fermentation Test

Buffer salt solution, (BSS) K_2HPO_4, 0.04g; KH_2PO_4, 0.01g; KCl, 0.8g; phenol red, 0.4ml 1 percent aqueous solution and distilled water, 100ml. This was stored at 4°C in a sterile glass bottle.

Twenty percent solutions of glucose, maltose, lactose and sucrose in distilled water were sterilised by membrane filtration and stored in 10ml amounts at —20°C. One bottle of each carbohydrate was thawed each week and kept at 4°C. 0.1ml of BSS was added to each of four tubes. 0.04ml of glucose, lactose, maltose and sucrose were added to individual tubes of the BSS. Each tube was inoculated with approximately a 2mm loopful of a pure culture of the organism from the subculture plate until a dense suspension was obtained. The inoculated tubes were incubated in a 37°C waterbath and examined at half hourly intervals for up to 4 hours. (A colour change from red to yellow signifies a positive reaction, an orange colour is regarded as negative).

Results

Table 1 shows the results obtained by the FA test and MRFT on 100 gram-negative diplococci isolated from genital and throat swabs.

Seventy-seven isolates were correctly identified as *N. gonorrhoeae* by the FA test and MRFT.

Two isolates had a negative FA test but were biochemically identified as *N. gonorrhoeae*. These were confirmed as being *N. gonorrhoeae* by National Health Institute, Wellington. There was only one strain that gave a positive FA test but appeared to be *Neisseria meningitidis* by the MRFT. The fluorescence was weak in this case and it was confirmed as *N. meningitidis* using CTA sugars.

Eight strains gave weak or doubtful fluorescence but when the FA test was repeated, became positive.

The MRFT had to be repeated on three strains when it was found that the sub-cultures were contaminated.

Discussion

The MRFT and FA techniques both appear to be reliable tests for the identification of *N. gonorrhoeae*.

The MRFT identified 100 percent of *N. gonorrhoeae* from cervical, urethral and oral cultures from the Venereal Disease Clinic, and the FA test identified 97 percent.

When immunofluorescent facilities are available the FA test is preferable as it is quick giving reliable clear cut results in most cases and can be performed on primary isolation cultures when growth may not be pure. Results are usually available after 24 hours incubation. However there are times when the fluorescence is doubtful and a confirmatory test is needed.

The MRFT was a suitable alternative as it gave reliable results and in a much shorter time than CTA sugars, usually 1-2 hours.

There have been reports of failure of strains of *N. gonorrhoeae* to grow in CTA media.[4] The method described depends on the presence of pre-formed enzyme and so the problem of growth does not arise.

Table 1.—Results of FA test and MRFT on 100 isolates

			MRFT			
Isolation Site	**No. of Isolates**	**Fluorescence**	**G**	**M**	**L**	**S**
Genital	77	+	+	—	—	—
	2	—	+	—	—	—
Throat	9	—	+	+	—	—
	1	+	+	+	—	—
	3	—	+	+	+	—
	8	—	—	—	—	—

\+ positive reaction
— negative reaction
G = glucose
M = maltose
L = lactose
S = sucrose

The only disadvantage was that a pure culture is necessary so the test could only be

done on subcultures and results were not available until after 48-72 hours. Contrary to another report,[5] we did not find that growth on GC Agar base plus haemoglobin and isovitalex was unsuitable for the MRFT.

The reagents for the MRFT have been shown to be stable on storage,[2] and will remain stable longer than carbohydrate culture media.

The Bacto FA *N. gonorrhoeae* conjugate has been said to cross react with *N. meningitidis*[3]. In the past this was not considered important as *N. meningitidis* is rarely isolated from genital specimens. However with the examination of oral cultures for *N. gonorrhoeae* increasing, it is important to be able to distinguish between these two species. In our experience we have had only one isolate of *N. meningitidis* that fluoresced with the FA gonococcal conjugate, which was the one in this survey. Twenty-one strains have been tested including the 10 in the survey and a further 11 strains from CSFs and throat swabs, all with negative results.

Isolates of gram negative diplococci from sites such as blood cultures, skin lesions, joints, and eye swabs require vigorous confirmation of identity and use of both FA and MRFT are recommended.

Summary

The MRFT would seem to be an excellent method for confirming the identification of *N. gonorrhoeae* that give negative or doubtful fluorescence with Bacto FA *N. gonorrhoeae* and a reliable diagnostic test for those laboratories lacking immunofluorescent techniques.

REFERENCES

1. Brown, W. J. (1974), *Appl. Microbiol.* **27,** 1027.
2. Brown, W. J. (1977), *Health Lab Sci.* **14,** 172.
3. Hare, M. J. (1974), *Brit J Vener Dis.* **50,** 437.
4. Pollock, Helen M. (1976). *J Clin Mic.* 4, 19.
5. Young et al (1976), *Brit J Vener Dis.* **52,** 172.

Evaluation of Two Inolex Products for the Identification of Members of the Family Enterobacteriaceae

M. D. McCarthy ANZIMLT, MASM

Medical Laboratory, 472 George Street, Dunedin

Received for publication, November 1978

Introduction

This paper is the result of an appraisal of two commercial test systems produced by the Inolex Corporation—Enteric 1 and Enteric 20—in comparison to the system currently in use in our Laboratory for the recognition and identification of members of the family *Enterobacteriaceae.*

This appraisal was carried out using 44 fresh clinical isolates and one stock culture.

Materials

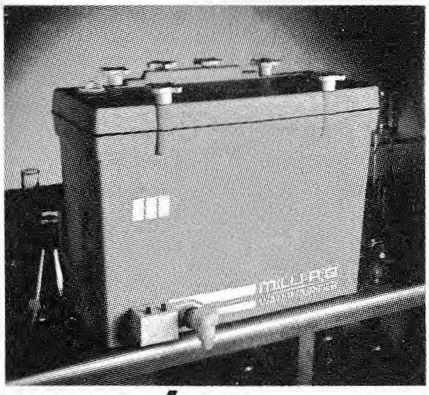
Inolex Enteric 20 Concept consists of the Inolex Enteric 20 card and the Var-ident 20 Binary Identification scheme.

The card consists of 20 test capillaries each containing a specific biochemical test substrate. When a suspension of the test organism is added to each capillary, the reagents react in a specific fashion resulting in a characteristic colour change.

The capillaries test the following reactions—malonate utilisation, glucose utilisation, phenylalanine deamination, B—galactosidase activity, indole production, hydrogen sulphide formation, lysine and ornithine decarboxylation, urease activity, sucrose fermentation, arginine hydrolysis, citrate utilisation and the fermentation of salicin, adonitol, inositol, arabinose, maltose, trehalose and xylose.

The glucose fermentation is linked with reazurin reduction and if the glucose is

SMALL STILLS JUST BECAME OBSOLETE.



The Milli-R/Q™ Water Purifier.

Consistently Better water.

The Milli-R/Q Water Purifier from Millipore will give you purer water than even double distillation. For a lot less than the cost of a single-stage still.

But better water and lower cost aren't the only reasons the Milli-R/Q Water Purifier will make small stills obsolete. For example, compare the maintenance requirements. A still needs weekly acid cleaning. Without it water quality deteriorates due to the increased carry-over of entrained boiler deposits. But with a Milli-R/Q you simply replace cartridges a few times a year (typically, every 3-4 months).

Smaller and more Economical

It takes up so little space, you can put it practically anywhere, on a shelf or counter top. And all installation consists of is connecting it to your faucet. The Milli-R/Q runs on tap pressure.

A comparably equipped still takes up to 3 times as much space and is almost double the price. Distillation also costs 2 to 3 times as much to operate. And it's extremely wasteful of heat energy and cooling water.

The Milli-R|Q Water Purifier.
The system that makes small stills obsolete.

The Milli-R/Q Water Purifier.
Better quality water than even double-distilled.

SMITH BIOLAB

P.O. Box 36007
Auckland 9, New Zealand.

Auckland	Ph. 483-039
Wellington	Ph. 683-453
Christchurch	Ph. 63-661

WITH RESIDENT REPRESENTATIVES IN HAMILTON, PALMERSTON NORTH AND DUNEDIN.

15040D

oxidised the electrons from the glucose reaction are transferred to the reazurin and this turns from blue to pink to colourless.

The Inolex Enteric consists of the Enteric I card and the Var-ident Binary Identification System.

The Inolex Enteric 1 card consists of 10 test capillaries and, as above, after the addition of the suspension of the organisms the characteristic reactions can be determined.

The Inolex 1 card consists of malonate, glucose and reazurin, phenylalanine ONPG, indole, H_2S, lysine and ornithine decarboxylase (separately), urea and sucrose.

For some isolates the organism may not be readily identifiable on the information from the Enteric I card and Inolex provide an additional Enteric II card, comprising the remaining 10 capillaries of the Inolex Enteric 20 card described previously.

API 2pE. In this laboratory the author has modified the Denver Laboratories API 20E system to the replicator technique but has used the API 20E Profile Recognition System in establishing the identity of the isolated organisms McCarthy (1973).[5]

Methods of Usage

All cultures were subcultured from CLED agar and particular care was taken to ensure that only one colony was sampled. This colony was touched with a sterile wire and the inoculum rubbed into 10ml of sterile saline. This saline was incubated at 37°C for 2-3 hours to ensure an adequate number of viable organisms in the inoculum prior to the inoculation of the card systems.

Each capillary is inoculated through the top hole and 5-6 drops of inoculum are needed to charge each capillary adequately. The capillaries for indole and phenylalanine are under filled to allow room for the addition of further reagents following incubation.

Each inoculated test card is incubated in its own moist plastic tray and cover at 37°C for 18 hours.

Reading of Results

Indole production: add 1 drop of Ehrlichs reagent to the test capillary and read for the characteristic pink colour of a positive reaction.

Phenylalanine production: add 1 drop of 10 percent Ferric chloride to the test capillary and read for the characteristic green colour of a positive reaction.

All other reactions are as conventional macro tests with the exception of the arginine dihydrolase. In this test system the medium becomes alkaline upon the deamination of the arginine and the colour change is from orange to a deep red.

Both the Inolex systems have their own individual charts for recording results.

Identification of Isolates

With the Inolex Enteric 20 the final identification is based on a computer-assisted system and the primary data base has been based on the extensive work of Edwards and Ewing (1972)[2] and Ewing (1973)[3].

The Inolex Codon is a 7 digit number developed from the card and produced by an octal number system. Briefly, the first 18 tests are grouped into six triplets, the first test in each has a value of 1, the second 2 and the third 4, whilst the final two tests are grouped together with the value of 1 and 2, respectively.

From the 7 digit number developed, the user refers to the Var-Ident 20 Manual for the listing of that Codon and will find that the majority of typical and moderately atypical organisms will show as a single entry to that particular Codon.

Occasionally, several organisms may appear as a multiple listing under that value and this indicates that the biochemical profile of the given organism will fit several different taxa. In this case up to four additional biochemical tests are recommended with the expected results for each taxon.

Improbable Test Results are also listed and these show, as a percentage, any test result that is significantly at variance from the expected result, for example, the test reaction was negative but the organism would be expected to have a positive reaction in more than 75 percent of tests; or the test reaction was positive with an expected positive result of less than 25 percent.

The Inolex Var-Ident system with the Enteric 1 card is based on the application of the

Boolian and binary code logic in which each positive biochemical test is assigned a value and each negative test is o. The assigned weights are:

Viability control (glucose and reazurin)	512	Phenylalanine	256
ONPG	128	Indole	64
H_2S	32	Lysine dec.	16
Ornithine	8	Urease	4
Sucrose	2	Malonate	1

The positive assigned weights are totalled and this number is then located in the Identification Manual. This Manual will indicate:

(i) When serotyping is necessary.
(ii) Further additional tests to separate several close taxa.

Results

Forty-four fresh isolates, mainly from urine specimens and one stock culture were inoculated through the system and the results recorded. These various numerical systems were applied and these codons checked in the Identification Manuals.

Thirty-four (77 percent) isolates were identified as *Escherichia coli* in the case of the API 20E and the Inolex 20 and as *Escherichia* in the Inolex Enteric 1 system.

Two (4.5 percent) were identified as *Proteus mirabilis* in all systems.

Two (4.5 percent) isolates were identified as *Klebsiella pneumoniae* in all systems.

This gives an absolute agreement of 86 percent (38/44) isolates.

Isolate 5602 gave an API Profile of 7104562 which was not recognised in the API Index—7104552 is listed, however, as *Arizona himshawii* 1:194 with the over-riding statement that 96 percent of isolates are H_2S positive.

The Inolex Enteric 20 gave a Codon 2211073—*E. coli* 1:219—and the Enteric 1 gave a Codon of 592 which does not appear on the Table. Both the Inolex systems agreed on the following positive biochemical reactions—glucose, indole, lysine, sucrose with the API 20 system indicating that the indole was negative, ONPG was positive, arginine and ornithine were positive.

Isolate 5797 gave an API 20E profile of 5144552 *E. coli* 1/7, the Inolex 20 2210073 *E. coli* 1/210 and the Enteric 1 576—a *shigella, Providencia stuartii* or *Prov. alcalifaciens* with additional tests required.

The tests in dispute between the Inolex 1 and the other two systems were the ONPG—positive in both 20 systems, and the lysine decarboxylase also positive in both systems. If this information was substituted in the Inolex Enteric 1 the code became 712—*Escherichia.*

Isolate 1111 was a stock culture of a non virulent *Salmonella typhi* and with API 20E gave a Profile 4004500 *Salmonella typhi* 1/20. The Inolex Enteric 1 gave an answer 528—glucose and lysine positive—*Salmonella* serology required.

The Inolex 20—with the same two positive findings—gave a Codon 2010003—*Shigella* species—serology required. The Inolex 20 Codon required positive reactions with sorbitol and maltose to give a Codon 2010053 for the recognition of *S.typhi* and this correlates with Edwards and Ewing 1972.

Isolate 7495 gave an API 20E profile 1044572 *E. coli* 1/11, Inolex Enteric 20 2301073 which showed two possibilities *E. coli* 1:191 and *E. agglomerans* 1:2283 and the indication that further testing with Jordans Tartrate medium and gelatin hydrolysis at 22°C would differentiate these taxa. The Inolex Enteric 1 gave a figure of 712—*Escherichia/Citrobacter diversus* with the additional information required from the Inolex 2 card—basically the citrate reaction. This was negative and the identification of *Escherichia* confirmed.

Isolate 7484 gave an API 20E Profile 5004573 *Klebsiella ozaenae:* Enteric 20 2301073 *E. coli* 1/191 *E. agglomerans* 1/2283 and Enteric 1, 1/722 *Escherichia.* The areas of difference were in the following tests—lysine decarboxylase positive API 20E and Enteric 1, indole positive both Inolex systems but negative with API 20E.

If the positive lysine result is transcribed to the Inolex 20 the Codon becomes 2311073:*E. coli* 1/24 and if the positive indole result is transcribed into the API 20E Profile the new profile becomes 5044573 *E. coli* 1/128.

Isolate 7398 gave an Inolex Enteric 20 Codon 626400 with the nearest number being 6264003 (i.e. fermentation of trehalose and xylose) *Proteus mirabilis* 1/2611 with the improbable results being indole positive and H_2S negative findings.

The Inolex Enteric 1 gave a numerical code of 588 and an acceptable identification of *Proteus morganii*.

The API 20E Profile was 0374000 *Proteus morganii* 1/55.

Isolate 7950 gave an Inolex Enteric 20 Codon 2231073 *E. coli* 1/122 with an improbable result as the negative ONPG. The API 20E Profile was 5144572 *E. coli* 1/28 with a positive ONPG. The Inolex Enteric 1 also gave a negative ONPG and the number 593 was not in the Var-Ident manual.

Discussion

The test system that gave the most variance in our hands was the ONPG in the two Inolex products.

A query to the Inolex Corporation was answered by V. L. Olson and he suggested that our reading of the test was not sensitive enough and that any trace of yellow colour is positive and the negative test is actually water clear.

He also commented that elevated temperatures in storage may adversely affect the performance.

All Inolex strips are sealed in a separate foil container with an individual sachet of desiccant in each strip.

Inolex also replied on the codon value obtained from the *Salmonella typhi* indicating the normally accepted results of positive findings for lysine decarboxylase, sorbitol and mannitol fermentation. If this product is to be widely used it may be worth commenting that this Codon should be reincubated for another 24 hours as *Salmonella typhi* may be the test organism.

The Inolex Enteric 20 data base is primarily a compilation of the data of Edwards and Ewing (1972)[2] and Ewing (1973)[3] which is based on a total of 16,261 cultures involving members of the family *Enterobacteriaceae*.

Inolex are supplementing these results and anticipate replacing or modifying the current Manual as significant data emerges.

Braune and Kocka (1975)[1] found the decarboxylase tests to be the more troublesome but that variation did not seem to apply to our series of organisms.

Conclusion

The Inolex Enteric 20 strip and the Inolex Enteric 1 strip were evaluated in this laboratory under normal working conditions.

Both strips are easy to use, no paraffin overlays are required, and, with the exception of the ONPG, give results comparable to the more established conventional test systems.

In a paper by Holmes *et al* (1978)[4] the author comments that "in considering different identification systems it is not the comparability of individual test results but the equivalence of the final identification which is important."

With this final consideration in mind the Inolex Enteric 1 and the Inolex Enteric 20 systems have proved quite successful in our hands to identify members of the family Enterobacteriaceae.

Acknowledgments

The material for testing was provided by Carter Chemicals, Dunedin from the Inolex Corporation, Biomedical Division USA.

REFERENCES

1. Braune, L. M., Kocka, F. E. (1975), *Med Microbiol Immunol.* **161,** 189.
2. Edwards, P. R., and Ewing, W. H., (1972), Identification of Enterobacteriaceae 3rd edition. Burgess Publishing Company Minneapolis Minn.
3. Ewing, W. H., (1973), Differentiation of Enterobacteriaceae by biochemical reactions. Revised. Centre for Disease Control. Atlanta, Georgia.
4. Holmes, B., Willcox, W. R., and Lapage, S. R., (1978), *J Chin Path.* **31,** 22.
5. McCarthy, M. D. (1973), *NZ J Med Lab Technol.* **27,** 49.

Microsporum nanum Infection of the Arm in a Golfer

Carol Bingham, ANZIMLT

Microbiology Department, Diagnostic Laboratory, Nelson

F. M. Rush-Munro, FNZIMLT

Mycology Reference Laboratory, National Health Institute
Wellington

Received for publication, February 1979

Summary

The identification of *Microsporum nanum* in the culture of scrapings from a lesion on the upper arm of a barman led to enquiries regarding his other activities. It was found that this comparatively rare cause of human ringworm had probably infected the patient, who was a keen golfer, when he retrieved balls from the rough ground of a piggery adjacent to the golf course. Subsequent attempts to isolate the dermatophyte from the soil and from brushings of some of the pigs were unsuccessful. This appears to be the fifth known case of human infection due to *M. nanum* in New Zealand.

Introduction

Microsporum nanum was first isolated in Cuba in 1954 from a scalp lesion of an eight-year-old boy. It was initially considered by Fuentes and his colleagues to be a dwarf form of *M. gypseum* and was described under the name of *M. gypseum* var. *nana*. Following the isolation of a second strain and the study of the two isolates, Fuentes described the new species as *M. nanum*. Further human isolates were reported from Mexico, the United States and Canada. In 1961 Dawson and Gentles reported the perfect state as *Nannizzia obtusa* by mating a Cuban isolate with one from Kenya. Although this latter strain came from a pig, it was not until 1964 that it was found that *M. nanum* was responsible for a common form of ringworm in pigs in the USA Ajello *et al.* (1964)[1].

Its presence in pigs in New Zealand was reported by Smith *et al.* (1966)[6] and the first human infection in this country was that of Baxter (1969).[2] Since that date only three human infections have occurred in New Zealand to our knowledge. Two of these were in the North Island, a boy from Gisborne, a woman from the Waikato area, and one from the South Island—a farmer from Marlborough. All three cases had had contact with pigs (Rush-Munro, unpublished). Morganti *et al.* (1976)[4] reported the first isolation from swine in Italy. They reviewed the reported human isolations in world literature and found only 19. The present case is reported to up-date the New Zealand isolations and to illustrate the indirect contact with pigs. From time to time there has been criticism of the necessity for identification of the specific dermatophyte responsible for a clinically characteristic ringworm lesion. This case stresses the epidemiological value of the cultural result. The probable origin of the infection was clearly indicated and the family pets could be exculpated. *M. nanum* appears to have been reported only once from a dog and so far not from cats.

Case History

The patient was a 26-year-old European male whose occupation was that of barman. He developed a circinate lesion 2.5cm in diameter on the outer surface of the upper arm. The lesion was not particularly inflamed, had a scaling surface with a raised edge clinically suggesting a typical ringworm. On enquiry it was found that the patient was a member of a country golf club which shares a small estuarine island with a pig farm. He had entered the piggery grounds to retrieve golf

balls on occasions prior to the appearance of the lesion but at no stage did he have direct contact with a pig. The infection was treated with tolnaftate (Tinaderm) and on re-examination some weeks later there was no sign of infection.

Laboratory Investigations

Scrapings from the lesion were collected for mycological investigation. A few fragments were mounted in potassium hydroxide and on examination showed the presence of characteristic dermatophyte hyphae (Figure 1). Further fragments were cultured on Sabouraud dextrose agar (BBL), mycosel agar (BBL) and lactritmel agar (Fort Richard) and incubated at room temperature. After a few days a white cottony growth became apparent on all media, later developing into a flat powdery buff-coloured thallus (Figure 2). The under surface was initially orange, deepening to reddish-brown with maturity. Microscopic examination of the mature fungus showed abundant one—to three-celled oval macroconidia. These were thin-walled and delicately roughened, borne singly on short conidiophores (Figure 3). Initially microconidia were observed as very rare clavate forms along the sides of the hyphae. The cultures were referred to the Mycology Reference Laboratory of the National Health Institute, Wellington. The thallus and macroconidia were found to be characteristic of *M. nanum*, Rebell *et al.* (1970)[5]. Microconidia were readily demonstrated in a cellotape flag preparation in lactophenol cotton blue under a coverslip. They were more numerous on potato dextrose agar (Figure 4). This technique obviates the delay of preparing slide cultures to verify the production of microconidia, Turner *et al.* (1976)[8]. These are not found in the *Chrysosporium* species which may resemble *M. nanum* and have been confused with it in the past, Ajello *et al.* (1964).[1]

Attempts were made to trace the origin of the infection by investigating soils and brushings from the animals. Three 100g surface soil samples were collected at the fence line separating the golf course from the pig farm. Brushings on sterile carpet squares (6cm × 6cm) were taken at pig level from fence wire, fence posts and nearby vegetation in the hope that a pig with ringworm might absentmindedly have rubbed against them. Several pigs had considerately chosen a position for sunbathing that was close enough to the fence to allow the cowardly to approach them without entering the piggery and risking bodily harm. Once the dust from the departure of the most nervous pigs had settled, it became apparent that two types remained within sampling distance. Those of the first kind obviously had an unpleasant sense of humour and these were left severely alone. Those of the second kind were more friendly and, as their ecstatic expressions indicated, were quite

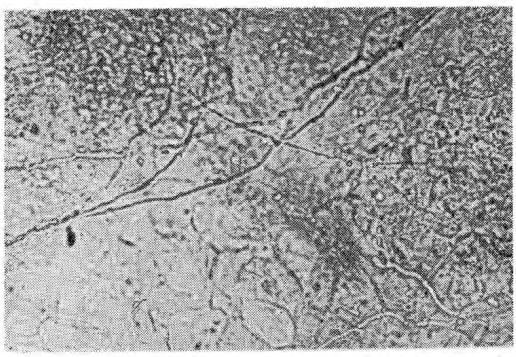


Figure 1.—Dermatophyte hyphae in skin from Lesion (x 450).

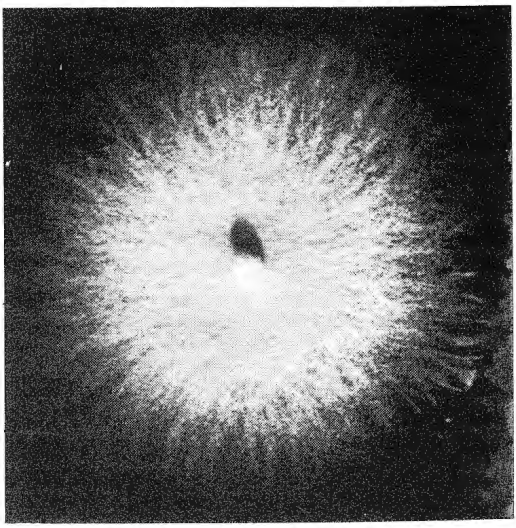


Figure 2.—Culture of *M. nanum* after four weeks on dermatophyte test medium.

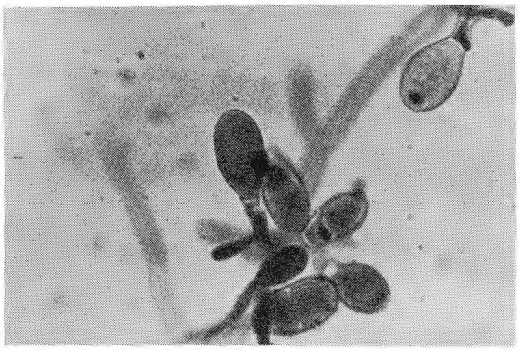


Figure 3.—Macroconidia of *M. nanum* showing septa and roughened walls (x 1000).

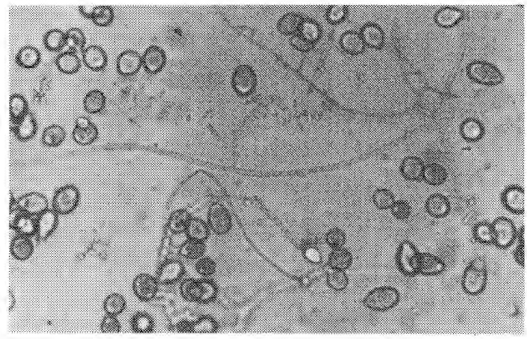


Figure 4.—Microconidia and macroconidia of *M. nanum* (x 450).

pleased to be brushed vigorously about the head and ears with a carpet square. This random sampling was carried out by character assessment rather than by the presence of possible ringworm lesions although one of the animals showed some scaling on the head. All samples were submitted to the National Health Institute for culturing.

Portions of the soil samples were placed in Petri dishes, moistened with sterile water and baited with short lengths (about 2-3cm) of sterilised child hair or horse hair (mane and tail hair are preferable). These were incubated for four weeks at 28°C and examined regularly for colonisation of the hairs. Further portions were cultured by the enrichment technique of Somerville *et al.* (1967)[7], which greatly increases the recovery of dermatophytes from soil. The soil was intimately mixed with sterile Keratin (nail clippings, hoof parings, hair clippings) moistened and held in closed plastic bags for 14 days before placing in Petri dishes and hairbaiting again as in the direct method. The carpet squares used to brush the pigs and their fomites were replaced in their paper containers and sent to the National Health Institute where they were cultured by pressing firmly on to the surface of dermatophyte test medium (DTM) dispensed in Petri dishes. These were also incubated for three to four weeks at 28°C and examined at regular intervals.

Results

No *M. nanum* or other dermatophytes were isolated from the soils, the pigs or their contact materials. A more comprehensive sampling of the piggery to determine the specific origin of the infection was not carried out as it was not considered that the area was an important focus of significant infection for the public.

Discussion

This unusual infection is reported to illustrate the value of culturing specimens from ringworm lesions in man. It demonstrates particularly well how fortuitous and transient may be the contact of the human with the animal host and its surroundings. Regrettably the causative fungus was not recovered from brushings of the few pigs sampled or from their environs which could be easily investigated. As in many similar episodes, the time-lapse between infection and sampling could be prejudical to success. The piggery is a "death-row" for the animals which emerge as pork products after a discrete time lapse. Members of the public are not considered to be at risk except by their own choice. There have been no cases of infection reported from piggery workers which seems a pity as this might give a condemned pig a slight sense of justice. It is possible that *M. nanum* is not easily contracted by man and infection may result from a slight abrasion or repeated friction with infective debris. It has been suggested that cases of ringworm due to *M. nanum* are relatively mild and short-lived. Such lesions could be disregarded or self treated by piggery workers and consequently medical advice is not sought, Baxter (1969)[3]. The notable exception to this clinical picture is the possibility

of tinea capitis in children developing into a kerion-type reaction. Fluorescence has been shown by some of the scalp infections under Wood's light. The invasion of the hair resembles that of *M. gypseum* with hyphae and sparse arthrospores on and in the hair shaft, Ajello *et al.* (1964)[1], Rebell *et al.* (1970)[5]. In their review of the geographical distribution of the 19 known human infections due to *M. nanum*, Morganti *et al.* (1976)[4] list the following countries: India, Australia, New Zealand, Cuba, Italy, Roumania, Canada, Mexico, United States and Brazil. It has been noted by Ajello *et al.* (1964)[1] that there is often no direct contact with pigs. The same authors reported the finding of the distinctive macroconidia in the soil of pig farms and hence classify the dermatophyte as geophilic.

Infection in pigs has been reported from Colombia, Egypt and Kenya in addition to the above countries where human infections were found. It is probable that infection in pigs occurs both from direct contact between animals and indirectly from soil of piggeries, Smith *et al.* (1966)[6]. It is considered to be rare in younger animals with transient inflammatory lesions but older pigs show a chronic condition involving large areas. These are frequently dismissed by pig workers as soil or faecal staining. There does not appear to be any hair loss.

Mycologically the fungus is readily cultured and identified. The observation of microconidia should be insisted upon to avoid the well known confusion with species of *Chrysosporium* which are frequently isolated from soils and from animal fur, Ajello *et al.* (1964)[1], Morganti *et al.* (1976)[4]. The larger spored members of this genus show roughened oval conidia, occasionally with a single septum but microconidia are not produced. Rebell *et al.* (1970)[5] warn of possible confusion with *Fusarium* species producing abundant rough-walled chlamydospores. Careful examination of these and the microconidia should clarify any difficulty. A further saprophytic soil fungus which tolerates actidione (cycloheximide) and may cause a misidentification is *Trichothecium roseum*. This species has a powdery pinkish thallus, (the colour is masked if isolated on DTM) and ovoid two-celled conidia are formed abundantly. As with *Chrysosporium*, no microconidia are produced and careful study of the conidiophore reveals the terminal clusters of conidia formed in basipetal succession.

Acknowledgments

The authors wish to thank Dr G. Kemble-Welch and Dr J. Ryder for permission to report the case history. We are indebted to Mr G. H. Bruggemans, Staff Medical Photographer, Wellington Hospital and to Mr A. J. Woodgyer, National Health Institute for the photographs. The technical assistance of L. Cain, R. Costello, S. Haycock and A. J. Woodgyer is gratefully acknowledged. Published with the authority of the Director-General of Health, Department of Health, Wellington.

Addendum

Two further cases of infection due to *N. nanum* have occurred recently. One in the Wellington area was on the dorsum of the right hand of an abattoir worker. He had apparently had minimal contact with pic carcasses which were said to have been scalded before handling. The second was from Nelson and again originated from the piggery on Best's Island. The patient lived on the island and passed through the piggery, opening and closing gates to reach his home. He had no direct contact with pigs. The lesion was on the outer aspect of the right shin. Both cultures showed easily detected microconidia.

REFERENCES

1. Ajello, L. Varsavsky, E. Ginther, O. J., and Bubash, G. (1964), *Mycologia* **56,** 873.
2. Baxter M., and Perason R. D., (1969), *NZ J Med Lab Technol.*, **23,** 87.
3. Baxter M., (1969), *NZ Med J,* **70,** 24.
4. Morganti, L., Bianchedi, M., Ajello, L., and Padhye, A., (1976), *Mycopathologia,* **59,** 179.
5. Rebell, G., and Taplin, D., (1970), *Dermatophytes, Their Recognition and Identification.* **26,** University of Miami Press.
6. Smith, J. M. B., and Steffert, I. J., (1966), *NZ Vet J,* **14,** 97.
7. Somerville, D. A., and Marples, M. J., (1967), *Sabouraudia.* **6,** 70.
8. Turner, T. W., and Kaminski, G. W., (1976), *Med J Aust,* **1,** 743.

Technical Notes

An Unusual Cryoglobulin

The occurrence of cryoglobulins in cases of paraproteinaemia is well documented and requires no review. The purpose of this communication is to report a case in which the physical form of the cryoprecipitate was most unusual. Normally the presence of a cryoglobulin in serum is detected by the appearance of a precipitate or of gelling of the serum, on cooling. In this case, cooling resulted in the appearance of a clear, water-white, relatively viscous liquid phase, underlying the serum. A reasonable comparison could be that of a serum contaminated with high density silicone oil.

The patient had a diagnosis of benign IgG paraproteinaemia with a paraprotein documented, without progressive rise over two years. Some peripheral vascular symptoms were present, but no cryoglobulin had previously been noted.

Some findings relevant to the cryoglobulin are as follows:

Temperature Effect

Serum separated at 37°C was held for 24 hours at various temperatures and then centrifuged.

37°C—No precipitate

22°C—No precipitate

14.5°C—Cryoprecipitate, approximately 5 percent by volume.

4°C—Cryoprecipitate, approximately 11 percent by volume.

Characterisation of Cryoglobulin

Electrophoresis of whole serum on cellulose acetate (Barbitone, pH 8.6) showed a discrete band in the mid beta-gamma globulin region. This band was markedly decreased in the cold separated supernatant serum and formed the predominant band present in the cryoprecipitate, which showed in addition, only a trace of albumin. Immunoelectrophoretic studies showed the paraprotein to be an IgG, with Lambda light chains.

On thin layer gel filtration (Sephadex G-200), the cryoglobulin appeared in the immunoglobulin region indicating a molecular mass of the same order.

Quantitation

Radial immunodiffusion gave the following quantitation:

	IgG	IgA	IgM
Whole Serum	30g°l	1.2g°l	0.45g°l
Supernatant Serum	14.9g°l	1.1g°l	0.45g°l

giving, by difference, a figure of 15.1g°l for the cryoglobulin concentration.

The washed cryoprecipitate had a total protein concentration of approximately 200g/l, by a biuret procedure.

Summary

A cryoglobulin is described which was present in serum at a concentration of about 15g/l. It is an IgG with Lambda light chains, which by electrophoresis on cellulose acetate migrate as a discrete band in the beta-gamma globulin region. Precipitation begins at 14.5—22°C and is virtually complete at 4°C. An unusual feature is the physical form of the cryoprecipitate i.e., a clear, water-white viscous liquid.

Acknowledgments

To Bill Beggs for the immunological studies, Robyn Ramsay who performed the electrophoresis and brought the phenomenon to my notice, and Medical Laboratory for permission to publish.

September, 1978.
G. F. Davis,
Medical Laboratory,
Auckland.

A New Haemagglutination Test for Hepatitis B Antibody

A new passive haemagglutination kitset is now commercially available[1] for the detection of Hepatitis B surface antibody (anti-HB_s). It can be used for the detection of anti-HB_s in

patients, although we have found that a radioimmunoassay (RIA) test[2] is more sensitive for this purpose.

The test system is designed for use in microtitre trays. However, we have adapted the procedure into Terasaki trays, thus greatly reducing the cost per test. Optimal detection sensitivity is achieved when sera are diluted 1:5. Our main application of this test has been in routine blood donor screening for acceptable titres of anti-HB_s. Plasma screened by this method are forwarded to Commonwealth Serum Laboratories (CSL) in Melbourne, Australia, for fractionation into Hepatitis B Immunoglobulin (HB_sIg) which is at present, in short supply.

The modified test is similar in technique to the "micro-Hepatest" method for the detection of Hepatitis B Antigen (HB_sAg)[3]. It is a simple and rapid procedure.

Method

A selected dilution of donor serum is made, based on the titre of anti-HB_s of a standard serum supplied by CSL. The dilution selected is calculated to detect anti-HB_s levels with a titre equal to or above that of the CSL standard. 2 μl of the reconstituted Hebsgencell sheep red cells are dispensed into the wells of a Terasaki tray and 2 μl of each serum dilution are added. The trays are mixed thoroughly by gently tapping the sides of the tray, then left in a horizontal position for 30 minutes. The trays are then tilted at 45° angle over a light box and read after 15 minutes. Suitable controls should be included. Reaction patterns are similar to the micro-Hepatest method.

The adaptation has been used routinely and has proved quite suitable for the detection of high titre anti-HB_s. The test also has a use in detecting anti-HB_s when the more sensitive RIA facilities are not available, and/or to provide an interim test result.

Acknowledgment

To Dr D. G. Woodfield, Director, Auckland Blood Transfusion Centre, for his helpful assistance.

R. A. N. Anderson,
Grade Technologist,
Auckland Blood Transfusion Service.
September 1978.

REFERENCES

1. "Hebsgencell", Green Cross Corporation, Japan. *Marketed by McGaw Ethicals*, Box 18-069, Auckland 6.
2. "Ausab", Abbott Laboratories, Chicago, Illinois.
3. Anderson A., Booth P, McLoughlin K, Skidmore P, Ford D. (1977), *NZ J med lab Technol.* **29**, 103.

A Micromethod for Measuring Human Chorionic Gonadotropins in Urine

In abnormal situations where a quantitative estimation, or titre of HCG is required a great deal of material can be used in finding the end point.

Suggestions for economising include timed agglutination of a latex particle test, Killip, (1974)[1] and using smaller amounts of reactants.

While the time method has been shown to work well, it is still necessary to find a dilution of urine which will react within the stated time interval. The static end point of the haemagglutination inhibition techniques can be clearly demonstrated and confirmed and they have an advantage in this respect.

A micro-adaption of the Luteonosticon Immunoassay Kit was described by McKay *et al.* (1974)[2] using the Cooke Laboratory Products Microtiter System and a lucite "U" plate. This was a straightforward micro-version of the standard technique and as the sensitised erythrocytes and HCG antiserum were separately packed this presented no problem.

The idea of using smaller volumes of the Pregnosticon "All-in" haemagglutination test presented itself. In this case both reagents are contained in the one ampoule. The standard technique is to add 0.1ml of urine, 0.4ml of water, shake for 1 minute and leave for two hours.

This was modified by adding 0.4ml of water to the ampoule, suspending the erythrocytes and pipetting 0.1ml aliquots into a microtiter lucite "U" plate using disposable 100 μl micropipettes. Suitable dilutions of urine, for example; neat, 1/5, 1/50, 1/100 were added from a 25 μl pipette dropper. The suspensions were mixed and left for 2h. The tests were read with a hand lens and the results were comparable with the standard method.

Suspension of the contents of the ampoule with water first, did not seem to interfere with

the subsequent agglutination process. Reaction of the HCG antigen and antibody must be fairly slow or the reaction favours the urine HCG.

A positive reaction was obtained with a pregnancy urine when aliquots of the suspension were removed from the ampoule at 30 minute intervals over two hours.

Smaller volumes could possibly be employed but the 25 μl Microtiter dropper and total volume were suitable for the apparatus.

A reduction to $\frac{1}{10}$ volumes that is 10 μl urine and 40 μl suspension in a lucite "V" plate was not successful.

The possible uses of the Microtiter technique includes serial dilution of urines with the capillary transfer pipettes and addition of the suspension. A number of urines could thus be processed in parallel.

Reasonable quantitation of HCG can usually be achieved with the use of two ampoules in sequence. The second series of dilutions can be interpolated at a point indicated by the first series of larger stepped dilutions.

REFERENCES

1. Killip, M. (1974), *Amer J clin Path.* **61,** 337.
2. McKay, E. J. and Christie, Barbara, A. (1974), *NZ J med Lab Technol.* **28,** 39.

February 1979,
R. D. Allan,
Chemical Pathology,
Dunedin Hospital.

Serum Cholesterol Determination on the IL Multistat III with the IL Test Cholesterol Kit

The laboratory has recently purchased a micro-centrifugal analyzer, the IL Multistat III, for which the manufacturer, Instrumentation Laboratory (IL), markets a range of clinical chemistry kits. The purpose of this note is to report the unacceptable performance, in particular the accuracy, of the IL Test Cholesterol Kit.

The IL method is essentially that of Allain *et al.* (1974)[1] and is calibrated using the serum-based liquid standard supplied in the kit. During comparison studies between the extraction FeCl acetic acid/sulphuric acid AA-1 method (Technicon, 1965)[3] and the enzymatic IL technique, it was apparent that the latter gave low values for control sera (Table). Similarly, low results were obtained for Lipidtrol (Dade), Serachol (General Diagnostics), SMA reference serum (Technicon) and Wellcomtrol Survey material (Wellcome Reagents Ltd). Patient samples (n = 101; range 3.4-9.5 mmol/l) showed only fair correlation (r = 0.908); the regression equation (concentrations in mmol/l) was y (IL method) = 0.896 × (Technicon method) + 0.508 and the standard error of estimate (Sy) was 0.54. These least-squares parameters suggest a proportional error of 10 percent, a constant error of 0.5 mmol/l and a random error of 0.54 mmol/l; thus a marked tendency to under-estimate high values and over-estimate low values.

Non-random errors (unless small) are obviously unacceptable and arise from calibration and methodology problems (Westgard and Hunt, 1973)[4]. This conclusion was confirmed by following the progress curves during the 10 minute reaction period. Neither patient samples nor control sera reached equilibrium (end-point) during the incubation. Reaction with the IL cholesterol standard, however, was rapid and essentially complete within 10 minutes. Chu and Turkington (1978)[2] have also observed differences in the equilibrium time between patient sera and control material.

Instrumentation Laboratory acknowledged that there is a problem with control sera and suggested that it arises from the lyophilisation process during manufacture of the control sera (personal communication). Further, IL now includes a card entitled "Control Related Limitations" in their cholesterol kits that warns of possible differences in equilibrium time between patient sera and control material; due to the lower activity of the cholesterol esterase towards cholesterol esters in control sera.

Whilst perfect correlation between chemical and enzymatic cholesterol methods cannot be expected, there are numerous reports in the literature confirming the accuracy of enzymatic methods. Hence, use of the IL Test Cholesterol Kit, as directed by the manufacturer, cannot be recommended.

Dispensette®, the quick-action "pipette" saves 90% of your time.

To be able to dispense straight from any original reagent bottle – this together with the further advantages of the Dispensette®, results in a minimum time-saving of 90% compared with conventional pipettes.

Dispensette®, a registered trade mark of the Brand Company

CARTER CHEMICALS LIMITED,
192-196 Castle Street,
P.O. Box 1326,
Dunedin, New Zealand.

Brand. First in the field.

BRAND

Control Serum	Assigned Value (mmol + l)	Table Number of Determinations	Mean Value Obtained	SD (mmol + l)	CV (%)
Elevated Lipids (Bio-reagents & Diagnostics)	7.9	24	5.71	0.32	5.6
Moni-trol I (Dade)	3.1	19	2.74	0.16	5.8
Moni-trol II (Dade)	5.75	19	4.75	0.17	3.6

REFERENCES

1. Allain, C. C., Poon, L. S., Chan, C. S. G., Richmond, W., and Fu, P. C. (1974), *Clin Chem.* **20,** 470.
2. Chu, S. Y., and Turkington, V. E. (1978), *Clin Biochem.* **11,** 70.
3. Technicon (1965) Method File N24a.
4. Westgard, J. O. and Hunt, M. R. (1973), *Clin Chem.* **19,** 49.

P. L. Hurst,
Chemical Pathology Laboratory,
Dunedin Hospital.
May 1978.

Abstracts of Papers NZIMLT Conference 1978

Transferable Antibiotic Resistance: Transfer Mechanisms and Epidemiology. M. D. Cooke, Cawthron Institute, Nelson.

Since the discovery of multiple antibiotic-resistant *Shigella* in Japan in the early 1950's, the world-wide occurrence of bacteria simultaneously resistant to many antibiotics has focused attention on the genetic elements responsible for resistance and their importance to medicine, public health, genetics and molecular biology.

The infectious multiple antibiotic resistance (R) factors are members of the class of extra-chromosomal genetic elements collectively known as plasmids. Similarities with other plasmids in the construction and mechanisms of transfer of R-factors will be discussed, followed by some epidemiological and ecological implications of R-factor transmissibility.

A Decade of Semi-synthetic Penicillins and Cephalosporins. H. C. W. Shott, Microbiology Department, Dunedin Hospital.

The impact of the cephalosporins and semi-synthetic penicillins was predicted by Sir Ernst Chain and other notable scientists. Reviewing the situation after more than a decade of clinical study some would say these therapeutic agents have been a mixed blessing. The object of the paper is to attempt to bring together certain clinical and laboratory aspects in order to obtain a perspective.

Chemotherapy of Primary Amebic Meningitis. J. J. Donald, R. T. M. Cursons, E. A. Keys, T. J. Brown, Department of Microbiology and Genetics, Massey University, Palmerston North.

Two strains of *Naegleria* and two strains of *Acanthamoeba* were tested *in vitro* for susceptibility to five chemotherapeutic agents. Minimum amebicidal and minimum inhibitory concentrations were determined in axenic culture using a semi-defined media. Chemotherapeutic agents which appeared effective were then tested against the amebae in a cell culture system.

Amphotericin B, which has been used successfully in the treatment of one case of PAM, is effective *in vitro* at a concentration of $0.54 \mu g.cm^{-3}$ against *Naegleria* species but has no effect on *Acanthamoebae*.

In contrast, 5-fluorocytosine and polymyxin B were ineffective against *Naegleria* species but were capable of inhibiting the growth of *Acanthamoebae* at concentrations of 10 and 63 $\mu g.cm^{-3}$ respectively.

The use of synergistic combinations was investigated using amphotericin B together with either rifampicin tetracycline or 5-fluorocystine for activity against *Naegleria* species. For *Acanthamoeba* species polymyxin B was used with either 5-fluorocytosine or tetracycline. The results of these combinations will be discussed.

The Human Metaphase Chromosome Method for Detecting dsDNA Antibodies and its Comparison with three Alternative Methods. M. W. Roberts, Immunology Department, Auckland Hospital.

Double-stranded DNA antibodies are considered diagnostic for Systemic Lupus Erythematosus, with a few exceptions. In our laboratory we have developed the Human Metaphase Chromosome (HM) method for the detection of these antibodies. Specificity and sensitivity has been verified by stain and enzyme treatment and we have found the HM method eminently suitable for measuring dsDNA antibodies.

More recently, we compared our results on 46 ANF positive sera by four dsDNA antibody detection methods. The methods were: The Human Metaphase Chromosome Immunofluorescent method (HM), Crithidia luciliae IF method (CL), Farr Radioimmunoassay (F) and a Haemagglutination kitset (HA). We evaluated the results by comparing each test with each other test in 2 × 2 contingency tables and determined the degree of association using the Chi squared test. Significant correlation was found between HM vs CL (18.1); HM vs F (4.5); though not with HA vs HM (0.5).

Our study shows the HM method gives comparable results to other recognised methods for detecting dsDNA anti-bodies. The HA kitset is of less value.

An Evaluation of the Technicon Automated Micro Complement Fixation Machine. T. R. Taylor, Microbiology Department, Hamilton Medical Laboratory.

Automated Complement Fixation Test (C.F.T.) machines have been in use for approximately 10 years. The purpose of this paper is to evaluate one of the latest of these machines; the Technicon Micro C.F.T. Analyser.

This machine uses the same basic principles as the manual C.F.T. method, with similar variations in procedure available to the operator, e.g., incubation time, sample rate and concentration, together with variations in concentrations of antigen, complement and cells.

Problems in setting up the machine together with the implementation of modifications for both economy of reagent usage and adaption to techniques other than Complement Fixation are discussed.

The National Health Institute Serology Proficiency Testing Programme. R. V. Metcalfe, Serology Laboratory, Department of Health, Wellington.

Over the last year the Serology Section of the National Health Institute has undertaken five proficiency testing programmes involving some 60 New Zealand and Pacific Island laboratories. Two of these programmes dealt with *Brucella abortus* serology and there was one each dealing with syphilis, toxoplasmosis and leptospirosis. Details were obtained from the participating laboratories of the different methodologies and reagents in use. The results of these programmes will be presented and discussed.

The Incidence of Serratia SPP in Clinical Specimens. H. J. L. Brooks, Department of Microbiology and Genetics, Massey University, Palmerston North.

Members of the genus *Serratia* were formerly considered to be nonpathogenic for man. In recent years, *Serratia* has been cited as an important cause for "opportunist-type" infections, particularly in the U.S.A.

A survey was undertaken in a diagnostic, bacteriology laboratory to determine the incidence of *Serratia* spp in clinical specimens. Although *Serratia* accounted for only a small proportion of enterobacteria-like isolates, it was considered to be the aetiological agent in

Transwab

The sterile swab system for transporting specimens from the patient to the laboratory..

simple as abc

- Completely immersed — not just moist like most of the others
- Choice of media
- Standard, E.N.T. & pernasal available
- Ready labelled for patient details
- Colour — coded for instant recognition

available from

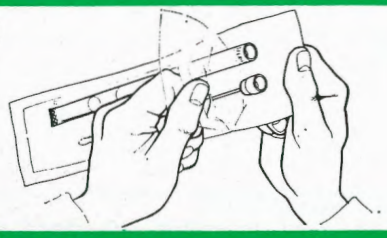
CARTER CHEMICALS LIMITED

P.O. BOX 1326, DUNEDIN
TELEPHONE 779-359

manufactured by

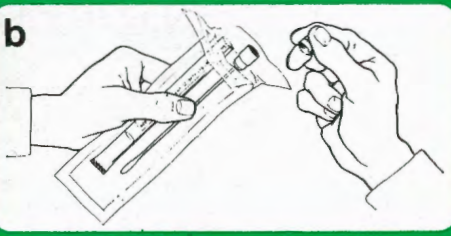
MEDICAL WIRE & EQUIPMENT CO (BATH) LTD.
Potley, Corsham, Wiltshire, England.

a



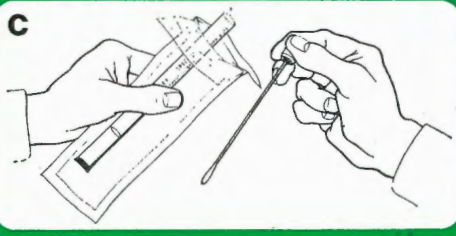
Peel to expose caps

b



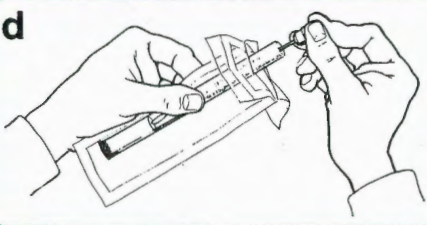
Discard transtube cap

c



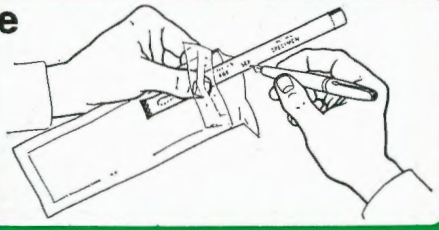
Collect specimen

d



Tube your applicator

e

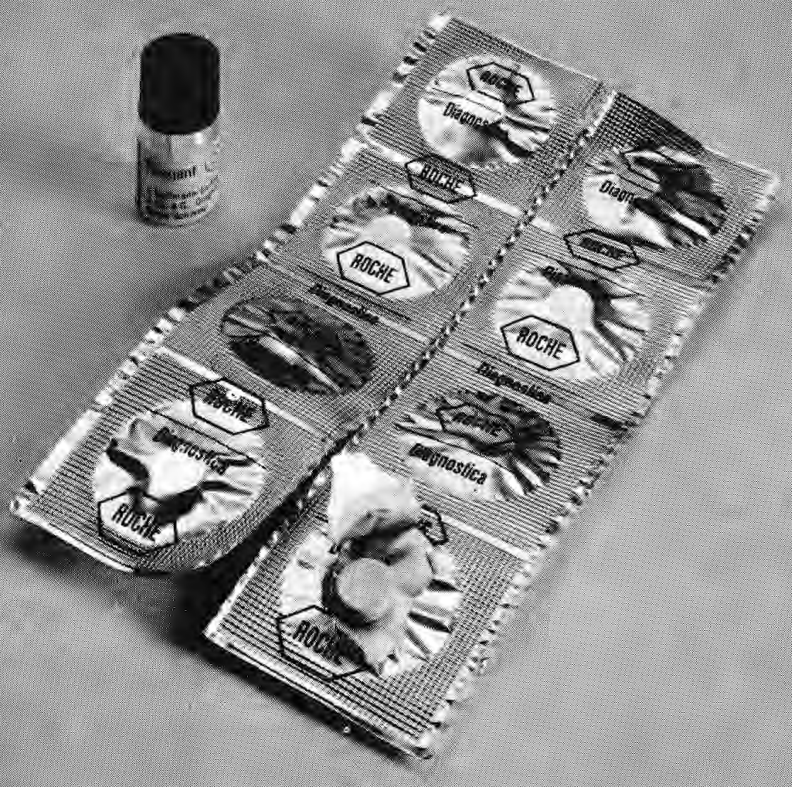


Fill in label.....& transport to the lab.

Triglycerides 'Rapid'

ROCHE Diagnostica

Fully enzymatic determination of triglycerides



The superior method – convenient and reliable

Roche Products (New Zealand) Ltd
P.O. Box 12-492
Penrose, Auckland

78-193

some cases of mild to severe infections. Serotyping and bacteriocine sensitivity tests revealed evidence of cross-infection, although the majority of *Serratia* infections were considered sporadic.

Pyuria of Uncommon Etiology. K. Fieldes, Microbiology Department, Christchurch Hospital.

A discussion of the investigations which can be carried out when routine culture for common bacterial pathogens is negative in the presence of persisting pyuria.

A case history of congenital urinary tract abnormality which presented as an acute urinary tract infection with septicaemia with an unusual bacterial cause, is given.

Microsporum Nanum Infection of the Arm in a Golfer. C. Bingham, Diagnostic Laboratory, Nelson and F. Rush-Munro, National Health Institute, Wellington.

This paper records the unusual case of a barman who developed a lesion caused by Microsporum nanum.

The lesion appeared after the patient, a keen golfer, had retrieved golf balls from a piggery adjacent to the golf course. Subsequent attempts to isolate the dermatophyte from soil and brushings were unsuccessful.

Acute Lymphoblastic Leukaemia Complicated by a Subcutaneous Aspergillus Infection. I. Wilkinson, Microbiology Department, Christchurch Hospital.

A case of aspergillosis of the skin, producing subcutaneous nodules on the arms and legs in a 19-year-old male, with a diagnosis of acute lymphoblastic leukaemia is presented, and demonstrating the difficulties encountered in the diagnosis.

Leprosy, and New Zealand in 1978. G. L. Cameron, Department of Microbiology, Wallace Laboratory, Auckland.

New Zealand has a large population of visitors and immigrants from areas where Leprosy is endemic and this has brought the diagnosis and treatment of this disease into the area of the clinical services.

Information about the acquisition and dissemination of Leprosy; the role of immunological processes in defining form of the disease acquired; and laboratory aspects of diagnosis and therapy control are presented.

Evaluation of Dynatech MIC 2000. G. J. Day, Department of Laboratory Services Wellington Public Hospital.

This paper compares a semi-automated identification and sensitivity testing method with the routine method at present in use at Wellington Public Hospital.

Practical Experience with Two Pye Unicam AC-30's. R. Law, Biochemistry Department, Middlemore Hospital.

The Biochemistry Laboratory at Middlemore has had two Pye Unicam AC-30's in use for approximately nine months.

This paper will outline the operation of the two analysers and worthwhile instrumental and methodology adaptations that have been undertaken.

Major advantages and disadvantages compared with other similar analysers will be discussed with some modifications made to the analysers which overcome most of the disadvantages.

The Estimation of Serum Iron and T.I.B.C. on the Abbott ABA 100. T. J. Lewis, Diagnostic Laboratory, Nelson.

A direct Bathophenanthroline method modified to suit the ABA 100 is presented. The reagent utilises Sodium lauryl sulphate to prevent precipitation of proteins. Because of the low serum to reagent ratio of 1:11, a separate blank run is necessary to correct for turbidity in the sample, but in spite of this a batch of up to 15 irons and T.I.B.C.'s can be completed in 30 minutes.

Obscurantism or How to Write a Paper. B. S. Collins, Waikpukurau Hospital.

Because most concepts of science are simple any ambitious scientist must, in self defence, prevent his colleagues from discovering that his ideas are also simple. All he must do is to write for publication so obscurely that no one

will attempt to read them but will be awed by erudition.

An Improved Direct Phosphorus Method. R. Law, Biochemistry Department, Middlemore Hospital.

The extremely sensitive Stannous Chloride/Hydrazinium Sulphate method has been modified for use on the Pye Unicam AC-30 analyser, the Technicon AA-1 Analyser and for manual analysis of serum and urine.

Moderate lipaemia contributes 0.02 mmol/l which is deemed clinically insignificant. Gross lipaemia has not been available for analysis. Bilirubin and Haemolysis do not interfere.

The working Stannous Chloride + Hydrazinium Sulphate reagent is stable for at least one week when refrigerated. Stock Stannous Chloride and Hydrazinium Sulphate solutions are stable for at least six months when refrigerated.

National Immunohaematology Proficiency Survey. R. J. Austin, Immunohaematology, Taranaki Base Hospital.

Forty-nine Immunohaematology Laboratories in New Zealand took part in N.I.P.S. (National Immunohaematology Proficiency Survey) in May and August of this year. Sixteen of these laboratories had taken part in two earlier pilot surveys.

This report presents for discussion the findings of the survey and attempts to explain the cause of some of the errors.

Comparison of Red Cell Antibody Elution Methods. Jan Hamer and Keiry Kennett. Read by K. McLoughlin. Immunohaematology Department, Christchurch Hospital.

A survey to compare the elution of 14 different human IgG antibodies against nine red cell antigens D Ce c E K Fy^a Fy^b Jk^a Jk^{ab}. The four methods used were Heat (Landsteiner), Ether (Rubin), Digitonin (Rosenfield and Kochwa), Acid (Rekvig and Hannestad).

Bovine Albumin as a Possible Cause of Reduced Reactions by A. H. G. Techniques. D. E. Roser, Immunohaematology Department, Dunedin Hospital.

The presence of inhibitors in Bovine Albumin is not a new phenomena. An apparent inhibitor was encountered in Dunedin following the introduction of a new Bovine Albumin into routine use. A comparative study was made with two other Bovine Albumins and some attempt made to identify the inhibitor, and its mode of action.

Piglets and Carpet Eating Children. M. Carolyn Reid, Haematology Department, Dunedin Public Hospital.

Pica, a common symptom of iron deficiency, is the compulsive eating of substances, usually a single item of food, ice or dirt, something within easy reach of the victim. A clinical history, laboratory results and discussion will be presented concerning iron deficiency and Pica.

The Middlemore Experience of Heparin Administration and Control. Miss C. Ellis, Haematology Department, Middlemore Hospital.

At Middlemore Hospital an enquiry was held into the administration and laboratory control of heparin therapy.

The paper discusses the A.P.T.T. test used to monitor heparin therapy, heparin storage and standardisation, the ward administration of heparin and individual response to heparin.

The results of the enquiry revealed many factors outside the laboratory that could influence the A.P.T.T. results.

The importance of laboratory and ward control through the medium of communication is emphasised.

Control of the Workload. R. T. Kennedy, Main Building Laboratory-Auckland Hospital.

It has been regularly stated that the laboratory workload rises at a rate of 10 percent to 20 percent annually. The reason for these increases should be questioned as they obviously lead to pressures within the laboratory service associated with expansion. The author believes that given a steady state within the hospital or population at large workloads should not significantly increase.

Several ideas are discussed, to bring the workload under control, ranging through data collection, application of cost benefit, investigation of traditional requesting patterns and budgetary constraints.

Iron Deficiency without Anaemia. T. J. Lewis, Diagnostic Laboratory, Nelson.

The investigation of all patients with a diagnosis of "tiredness, fatigue, listlessness, etc" should include estimation of serum iron and T.I.B.C. In our experience there are many cases of iron deficiency without anaemia. These patients have increasing tiredness which is relieved by administrating of iron. Several case histories will be presented to illustrate the above.

Lymphoproliferative Disease with Increased IgM Macroglobulins. Brett P. Tapper, Tauranga Medical Laboratory.

Lymphoproliferative Disease is relatively common, and, as such ordinarily would not warrant discussion at this forum. However, there are some interesting possibilities which may be associated with Lymphoma type disease, of which macroglobulin is one.

Mr S. has a disease process dating from as early as 1963 and for the greater part of his history has been producing abnormal amounts of IgM paraproteins.

This, then, is a case of Lymphoproliferative disease a little out of the ordinary.

Human Resources and Management in Medical Laboratories—An Overview. G. F. Lowry, Medical Unit, Princess Margaret Hospital, Christchurch.

Management, as distinct discipline in the medical laboratory environment, is currently undergoing a mixed reception around the world. This is particularly so in relation to the laboratory scientist, and the recognition of the vitally important role of the first line supervisor, e.g., Charge Technologist.

The management of people, their level of morale and motivation, are unquestionably key factors in gauging the success or otherwise of any enterprise, and the ability to realise objectives set by the Laboratory Directorate.

This paper attempts to emphasise some of the basic management concepts, both historical and of the present day, which contribute not insignificantly, towards attaining both organisational and personal goals.

Management by Objectives in the Laboratory. P. McLeod, Laboratory, Nelson Hospital.

Although no longer considered to be a new form of management, the concept of Management by Objectives has now become one of the most accepted and successful systems of management. Some of the characteristics of Management by Objectives include:

1. The total involvement of all staff at all levels in the decision-making processes.
2. Goal setting.
3. Appraisal of performance.

This paper will hopefully demonstrate the need for a management system in the laboratory. Also it will describe the system of Management by Objectives and suggest how it can be used in such an area. The Canadian Workload Uniting System has now given us a reliable and uniform means of measuring the output of the laboratory. This data can now be put to a variety of management uses as well as being used in the overall management system of Management by Objectives.

Correspondence

Sir,—We read with interest your recent article on locally manufactured sensitivity discs (Bragger and Heffernan NZ J med lab Technol. **32,** 3). We have recently completed a similar investigation in this laboratory, and the results obtained confirm those of the authors.

From one manufacturer we tested for uniformity within batches of Erythromycin, Tetracycline, Gentamicin, Sulphonamide, Pencillin Ampicillin, Trimethoprim, Cephalothin and Nitrofurantoin discs. Each batch tested showed a variation in zone size greater than

the 2.5 millimetres maximum as quoted in the article. In some cases, the variation from the smallest to the greatest zone was as much as 8 millimetres. We also noted a significant number of eccentric zones.

We also assayed discs from both manufacturers for potency, using zone measurement, and elution techniques. We assayed Penicillin, (2 unit) Ampicillin (25 micrograms), and Carbenicillin (100 micrograms) discs.

Both makes of Carbenicillin discs were within the limits outlined in the article. However, with Penicillin, discs from one manufacturer contained 300 percent of the stated dose and with Ampicillin, discs from the other manufacturer contained 160 percent of the stated dose. These figures represent average dosages for the batch. Similar results were recorded by both methods of testing.

From these results we can only agree with the authors, that locally manufactured discs have a lot to be desired with regard to uniformity of impregnation, and to accuracy of disc content.

G. L. Cameron,
I. Te Wiata,
Microbiology Department,
Auckland Hospital.

Book Reviews

Biotyping in the Clinical Microbiology Laboratory. Edited by Albert Balows and Henry D. Isenberg. A monograph in the Bannerstone Division of American Lectures in Clinical Microbiology, 1978, 116 pages, illustrated. Published by Charles C. Thomas, Springfield Illinois, USA. Price: $USA11.00.

This monograph attempts rather well to place in proper perspective the need for biotyping and how it can be approached in any competent microbiology laboratory. Biotyping according to these authors encompasses not only biochemical grouping of organisms but also; antigenic structure, DNA relatedness, antibiograms, phage susceptibility, response to bacteriocins, and measurement of metabolic end products. The book attempts to enthuse the reader either to begin a programme of biotyping or to improve (update) an already existing programme. It is by no means a complete treatise on the subject but does explain what biotyping is about, when it is best employed and for what reasons.

The book does not mention such procedures as phage typing of *Pseudomonas aeruginosa,* a typing procedure that "finger prints" 99 percent of these organisms—serological reactions and bacteriocin typing only are mentioned, nor do the clinically important and readily biotypable Lancefield Group D streptococci rate much mention. Lancefield Group A and B streptococci are presumptively identified using Bacitracin sensitivity and the CAMP test respectively, both basic typing procedures which do not rate a mention in this monograph.

Chapter 6, entitled "Biotyping of Anaerobic Bacteria associated with Disease" is essentially concerned with *Bacteroides* species and pays only lip service to the clostridia and other anaerobes despite the all embracing chapter title.

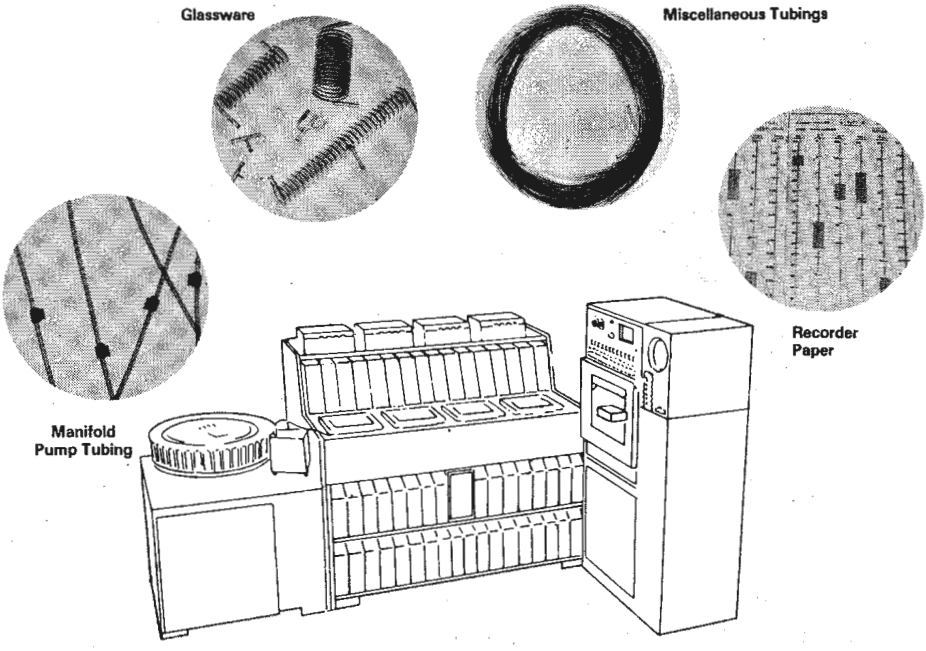
Chapter 7, on standardisation and automation in biotyping is particularly enlightening with many photographs of equipment available—at least in America. Each chapter contains a number of references which the reader should find useful.

One still has to weigh the cost of such a programme, with its value in terms of real benefit, in always needing to know the exact identity of a particular micro-organism. This is an American monograph and I for one would find some of the fairly extensive biotyping procedures outlined a little difficult to justify always. Nonetheless this volume would be a very useful adjunct to any progressive microbiology laboratory.

G. S. Elliott.

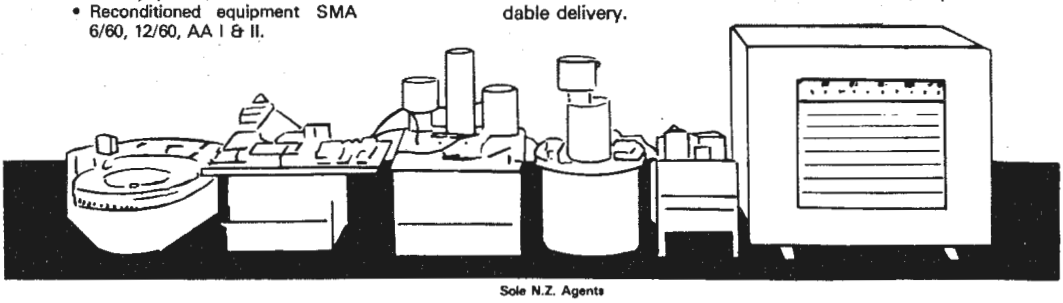
NORWOOD

"One source for all your auto analysis needs"



- Manifold Tubing Standard, Solvaflex & Flow-rated.
- Glassware (all types)
- Dialyzer plates & blocks
- Membranes • Stream Dividers
- Motor Stirrers • Nipples
- Pen Holders and Fiber tip pens
- Labels & tapes
- Chart paper • Connectors
- Reconditioned equipment SMA 6/60, 12/60, AA I & II.

Throughout the world, wherever there are hospitals and laboratories using Auto-analysis, NORWOOD laboratory products are used. NORWOOD has earned a reputation for quality and dependability at realistic prices. That's why more and more hospitals and laboratories are specifying NORWOOD. They want quality, dependability and reasonable cost. They get it from NORWOOD. NORWOOD distributors offer fast, dependable delivery.



NORWOOD SCIENTIFIC ASSOCIATES INC., New Jersey, USA.

Sole N.Z. Agents

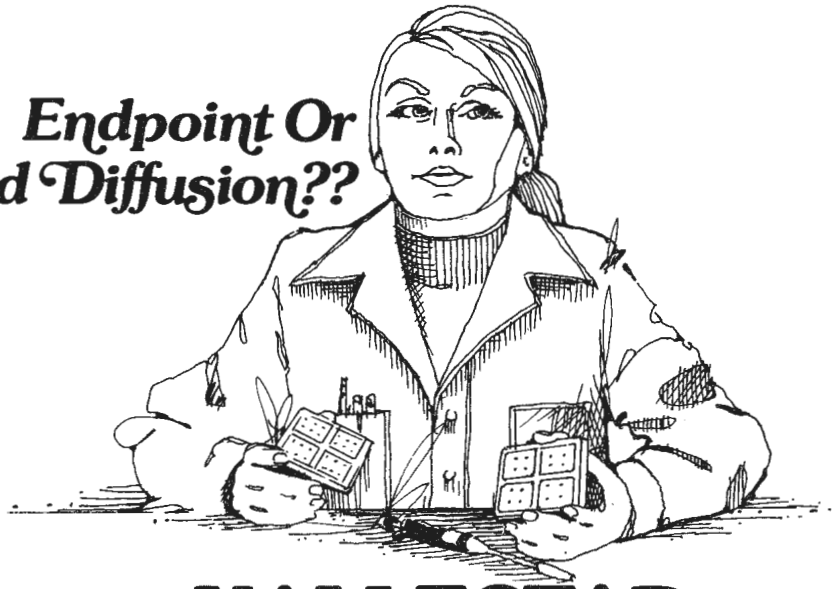
GENERAL DIAGNOSTICS (NEW ZEALAND) LTD.

206 THE PARADE, ISLAND BAY, WELLINGTON, 2. P.O.BOX 7170 WELLINGTON SOUTH. CABLES & TELEGRAMS: DIAGNOSTIC. TELEX: GENDIAG NZ 3859. TELEPHONE 836-255

RADIAL IMMUNODIFFUSION

RADIAL IMMUNODIFFUSION
RADIAL IMMUNODIFFUSION

Endpoint Or Timed Diffusion??



KALLESTAD OFFERS BOTH

NO PATIENT DILUTIONS for immunoglobulin quantitations with **ENDOPLATE** (endpoint) and **QUANTIPLATE®** (timed diffusion) Test Kits

ROOM TEMPERATURE INCUBATION — no moisture chamber or 37°C incubator required

EIGHT DIFFERENT PLATE CONFIGURATIONS — to fit your laboratory needs

MOST COMPLETE QUALITY CONTROL SYSTEM with each kit — to assist your laboratory's quality control program

PREDILUTED REFERENCE SERA with each kit **INCLUDING LOW LEVELS** — for your convenience

SMITH BIOLAB SCIENTIFIC DIVISION

P.O. Box 36007,
Auckland 9, New Zealand.

Auckland	Ph. 483-039
Wellington	Ph. 683-453
Christchurch	Ph. 63-661

WITH RESIDENT REPRESENTATIVES IN HAMILTON, PALMERSTON NORTH AND DUNEDIN.

150408

The Principles and Practice of Blood Grouping. Addine C. Erskine, Wladyslaw W. Socha. 2nd Edition 1978. Published by The C. V. Mosley Company, St Louis and obtained from N. M. Peryer, C.P.O. Box 833, Christchurch. 424 pages, illustrated. Price $NZ26.55.

This reviewer is not familiar with the first edition of this book and so was able to carry out the review without any preconceived ideas as to the value of the current text.

This publication is divided into three parts. 1. Principles; 2. Blood Groups of Nonhuman Primates and; 3. Methods. An adequate glossary is also included.

The paper used is of a glossy nature which I found distracting occasionally if the book was held at a certain angle to the light. Throughout the text bold type is put to good use to draw the reader's attention to particular words or headings. Each chapter is followed by a list of references plus a list of recommended further reading, the latter being rather useful. Certain sections of the text I found to be rather repetitive.

It is quite obvious from the outset as to where the authors' loyalties lie, photographs of Landsteiner, Levine and Wiener grace the frontispiece.

This publication I felt would not be readily accepted in New Zealand as the nomenclature used throughout is that of Wiener. For example the authors refer to the antigen and antibody C associated with the ABO blood group system. Also naturally prominence is given to the Wiener theory of the Rhesus system and only a passing reference is made to the theories of Fisher, Race and Rosenfield. It is clear from reading the text that the authors are wholly sold on the Wiener theories and everything else is either incorrect or has not been proved. The LW antigen is not associated with the Rhesus system according to the authors and is therefore featured in a chapter on high and low frequency antigens. A total of 15 pages is devoted to the other important blood groups systems excluding MNS which is given a chapter to itself indicating the work that Wiener carried out on this system.

Two chapters are devoted to the theory and methodology of blood grouping of apes and monkeys.

Generally I can find no reason to recommend this book for routine use in the Blood Banks of New Zealand. It may serve some useful purpose where a technologist is contemplating emigration to the United States and wishes to familiarise him or herself with North American terminology or is considering carrying out a blood grouping survey in the monkey house of the Auckland Zoo.

A. E. Knight.

Abstracts

Contributors: E. R. Crutch, Shirley Gainsford, Ainslie Langford, N. Langford and L. M. Milligan

Clinical Biochemistry

An Interfacing Factor in the Automated Analysis of Calcium. Gosling, P. and Sammons, H. G. (1979), *J. clin. Path.* **32,** 113.

Stainless steel components, such as probes and connectors within sampling systems of autoanalysers, that come into contact with sera, controls and standards, can act as pH dependent ion exchangers, absorbing calcium under alkaline conditions and under acidic conditions releasing previously absorbed calcium. The use of polypropylene is found to eliminate this source of error.

—N.L.

Immobilised Enzymes in Continuous-Flow Analysis. Werner, M., Mohrbacher, R. J., Riendeau, Carol J., Murador, E., and Cambiaghi, S. (1979), *Clin. Chem.* **25,** 20.

Glucose, Urea, and Uric Acid are assayed using Glucose oxidase, Urease, and Uricase immobilised onto the inner surface of polyamide tubing. Each enzyme bearing tube could be used for several thousand tests on both conventional continuous flow systems and hybrid micro-scale systems comprised of modules of different manufacture.

—N.L.

Improved Radioimmunoassay of Urinary Estriol. Jawad, M., Wilson E., and Kincaid, H. (1979), *Clin. Chem.* **25,** 99.

A radioimmunoassay protocol for estriol that is unaffected by glucose is described. 20 μl of urine is hydrolysed for 30 mins. and assayed by a rapid double-antibody radioimmunoassay with a total incubation time of 55 minutes. The assay is unaffected by glucose and correlated directly with a colour-metric assay based on the Kober reaction.

—N.L.

Detergent-Containing Glucose Oxidase for Use with the Beckman Glucose Analyser. Bajema, L., Lee, W., Zebelman, A. and Margaret A. Kenny (1979), *Clin. Chem.* **25,** 127.

The preparation of a glucose oxidase reagent for use with the Beckman "Glucose Analyser" that is less expensive than the commercially prepared reagent is described. Using Brij 35, instead of glycerol which has been shown to cause precipitation in reagent transmission tubing, maintenance time has been reduced to that recommended by the manufacturer.

—N.L.

Interpretation of Serum Total Calcium: Effects of Adjustment for Albumin Concentration of Frequency of Abnormal Value and on Detection of Change in the Individual. Payne, R., Carver, Margaret E., and Morgan, D. B. (1979), *J. clin. Path.* **32,** 56.

After measuring Total Serum Calcium in 1693 patients over a four-month period, it is concluded that adjustment of the Serum Calcium concentration for Albumin is essential to detect and assess changes in abnormal values.

—N.L.

Sequential Changes of α_1 Antitrypsin after Surgical Trauma. Lamy, Yvonne, Shawki, I., Lomanto, C., and Dombrowiecki, A. (1978), *Clin. Chim. Acta.* **89,** 387.

A significant drop in the serum level of α_1 antitrypsin preceding the rise known to occur after surgery is reported. Eighty patients were studied, with three samples taken from each, before, during, and 48 hours after surgery. The decrease in serum levels was independent of age, sex, type of surgery or anaesthetic. The cause of the decrease is postulated.

—N.L.

Automated Enzymatic Assay for Plasma Ammonia. Humphries, B., Melnychuk, M., Donegan, E. and Snee, R. (1979), *Clin. Chem.* **25,** 26.

The determination of plasma ammonia by an enzymatic method on the Du Pont Automatic Clinical Analyser (aca) is described. The assay involves the condensation of ammonia and α-ketaglutamate catalysed by glutamate dehydrogenase. NADPH is oxidised to NADP and the rate of decrease in absorbance at 340 nm is directly related to the ammonia concentration. Use of NADPH instead of NADH as the coenzyme eliminates the pre-incubation time usually required for endogenous dehydrogenase reactions. All reagents required are contained in the analytical test pack and this self-contained reagent system minimises extraneous ammonia contamination.

—A.L.

Double Light-Chain Disease: A Case Report. Dalal, F. and Wcsten, S. (1979), *Clin. Chem.* **25,** 190.

This case history deals with the discovery of a patient demonstrated to have both lambda and kappa monoclonal light chains in urine. It was found that the light chains were separate, distinct and not part of the immunoglobulin molecules such as IgG, IgM, IgA, IgD or IgE. Column chromatography confirmed these findings.

—A.L.

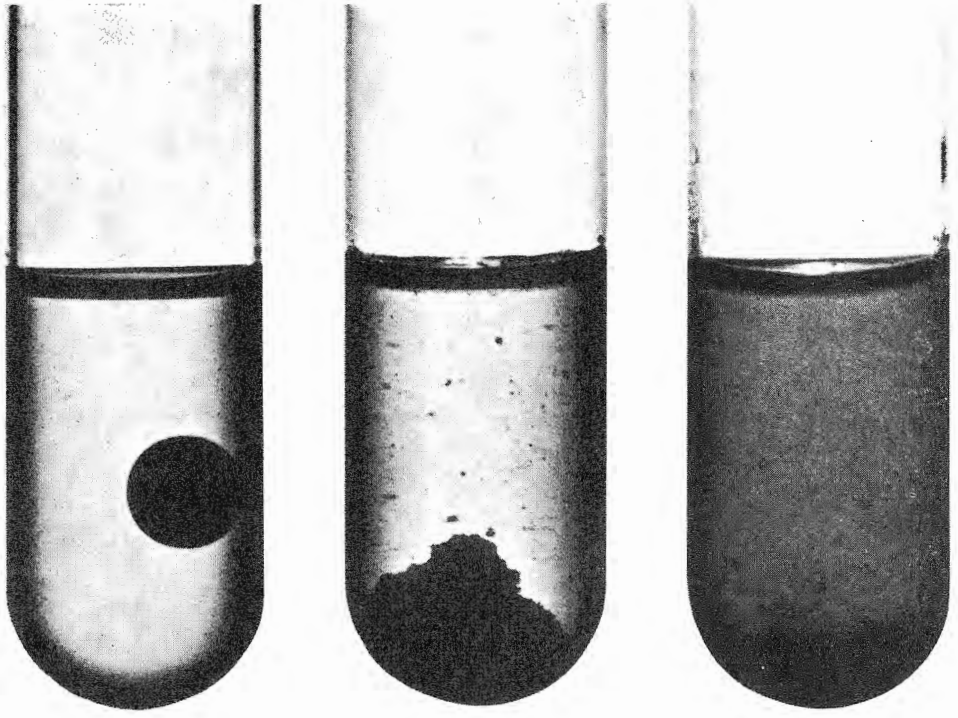
ROCHE Diagnostica

A progress

Determination of α-amylase with ‹Amylochrome› Roche

International Units

Dye bound covalently to amylose
instead of starch as substrate



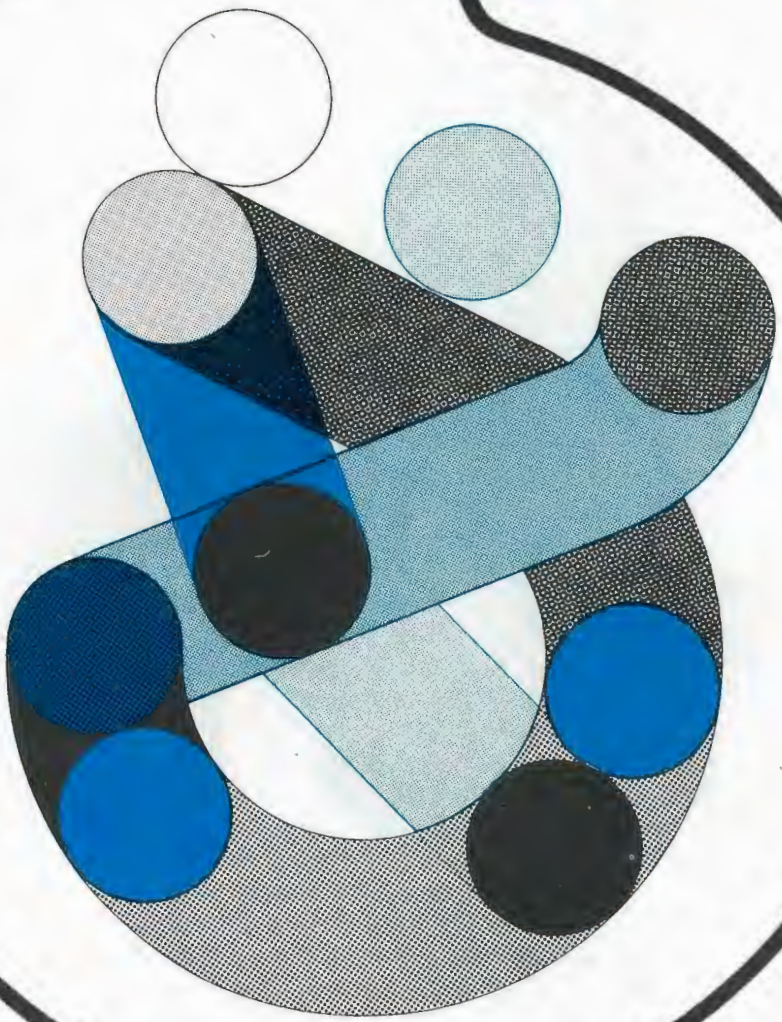
Amylochrome = Trade Mark

Roche Products (New Zealand) Ltd.
P.O. Box 12-492, Penrose, Auckland

78-183

Fluka's here to stay

Fluka research chemicals have been invited back, again and again to hospitals and laboratories all over New Zealand. Fluka research and laboratory chemicals have set the standard. Orders are telexed weekly and delivered by air thirty days from receipt of an order.



CARTER CHEMICALS LIMITED

Carter Chemicals Limited
P.O. Box 1326, Dunedin
Telephone 779-35

On-line Continuous Potentiometric Measurement of Potassium Concentration in Whole Blood During Open-Heart Surgery. H. F. Osswald, R. Asper, W. Dimai and W. Simon (1979), *Clin. Chem.* **25,** 39.

A flow-through system for the measurement of blood potassium is described, using an ion-selective electrode. This measurement is performed continuously and on-line off the extracorporeal blood circulation during open-heart surgery. With the advent of continuous measurement, the time delay involved with flame photometry analysis is eliminated. A comparison has been made with other potassium measurements made by the SMA flame photometer and the Orion SS-30 sodium and potassium analyser.

—A.L.

An Approach to Quality and Performance Control in a Computer-assisted Clinical Chemistry Laboratory. Undrill, P. E., and Fraser, S. C. (1979), *J. clin. Path.* **32,** 100.

This paper describes a comprehensive computer-based laboratory system which has been in operation since 1970. Details include performance and quality control techniques assessed by analysis of results from fixed-level control sera and cumulative sum methods. The computer system consists of a Digital Equipment Co. Ltd. PDP12 and a PDP8 + F computer.

—A.L.

Evaluation of Two Commercially Available Laser Nephelometers and Their Recommended Methods for Measurement of Serum Immunoglobulin. G. R. L. Walsh, Margaret E. Coles, and T. D. Geary (1979), *Clin. Chem.* **25,** 151.

Two laser nephelometer systems. Hyland and Behring, are described for the assay of specific proteins. The overall performance of the systems was assessed by using immunoglobulin G. Discussion includes accuracy, linearity, ease of operation, standard curve stability and accessory equipment.

—A.L.

Haematology

"On Line" Data Handling in a Routine Haematology Department Burmester, H. B. C., and Crow, G. S. (1979). *J. clin. Path.* **32,** 254.

A stand alone "on line" mini-computer complete with data handling procedures is described, which by utilisation of an associated microprocessor, enables the department to be totally independent of all manual paper work.

—E.R.C.

A Comparison of Five Manually Operated Coagulation Instruments. Beckala, H. R., Leavelle, D. E. and Didisheim, P. (1978), *Am. J. clin. Pathol.* **70,** 71.

The five instruments evaluated were (1) the single Channel Coag-a-Mate (General Diagnostics), (2) the Coagulyzer Jr (Sherwood), (3) the Clotek (Hyland), (4) the Electra 650 (Medical Laboratory tutoration) and (5) the Fibrometer (B.B.L.). A one month study was carried out on these instruments with prothrombin times and activated partial thromboplastin times.

—E.R.C.

Standardisation of the One-Stage Assay for Factor VIII. Zacharski, L. R., and Rosenstein, R. (1978), *Am. J. clin. Pathol.* **70,** 280.

The purpose of this paper is to elucidate certain aspects of this assay that have not received adequate consideration and to describe the authors' assay method in order to provide a basis for comparison with results from other laboratories.

—E.R.C.

The Dilute Whole Blood Clot Lysis Assay: A Screening Method for Identifying Postoperative Patients with a High Incidence of Deep Venous Thrombosis. Comp, P. C., Jacocks, R. M., and Taylor, F. B. Jr (1979), *J. Lab. Clin. Med.* **93,** 120.

A dilute whole blood clot lysis assay was used in comparison with the ^{125}I-fibrinogen leg scan. Very good correlation was obtained showing that the clot lysis assay does provide a means of identifying a group of postoperative

patients who have a high incidence of thrombophlebitis.

—E.R.C.

Serum Ferritin in Pregnancy. Jenkins, D. T., Wishart, M. M., and Schenberg, C. (1978), *Aust. NZ J. Obstet. Gynaec.* **18,** 223.

The results obtained from 200 consecutive pregnant women showed that 32 percent had low serum ferritin levels. This paper also shows that serum ferritin is a more sensitive indicator of the iron status of a patient than serum iron or haemoglobin levels.

—E.R.C.

Leukaemic Reticuloendotheliosis. (Hairy Cell Leukaemia). Bouroncle, Bertha, A. (1979), *Blood.* **53,** 412.

The author of this paper discusses the clinical, haematologic and histologic findings in 82 patients followed for over 20 years with Hairy Cell Leukaemia.

—E.R.C.

Myeloperoxidase Cytochemistry Using 2, 7-Flurendiamine. Benavides, I. and Catovsky, D. (1978), *J. clin. Path.* **31,** 1114.

The myeloperoxidase cytochemical reaction has been used as a tool to help distinguish acute myeloid leukaemias from acute lymphoplastic leukaemias. The most common substrates used are Benzidine derivatives, but they have major carcinogenic problems. 2, 7-fluorenediamine has been found to be an adequate substitute.

—E.R.C.

Recommendations of a System for Identifying Abnormal Haemoglobins. International Committee for Standardisation in Haematology (1978), *Blood* **52,** 1065.

This paper details the procedures and methods recommended by the International Committee for Standardisation in Haematology for identifying abnormal haemoglobins, including the Screening laboratory, Reference laboratory and Structure laboratory.

—E.R.C.

An Artificial "Haemophilic" Plasma for One-Stage Factor-VIII Assay. Chantarangkul, V., Ingram, G. I. C., Thorn, M. B. and Darby, S. C. (1978), *Br. J. Haemat.* **40,** 471.

An "artificial" plasma for one-stage factor-VIII assays is made by incubating human plasma with E.D.T.A., to destroy factor VIII, and afterwards removing the anticoagulant by dialysis. Bovine factor V is then added to a given level.

—E.R.C.

Comparison of Reagents for Determining the Activated Partial Thromboplastin Time. Hoffmann, J. J. M. L., Meulendijk, P. M. (1978), *Thrombos. Haemostas. (Stuttg.)* **39,** 640.

Six commercially available reagents for the determination of the activated partial thromboplastin time were evaluated and compared with respect to their sensitivity to factors VIII, IX and XI and their response to heparin.

I would suggest that all laboratories using commercial activated partial thromboplastin time reagents should note the results and conclusions drawn in this paper.

—E.R.C.

Immunohaematology

Methods of Assessing Factor VIII Content of Stored Frozen Plasma Intended for Preparation of Factor VIII Concentrates. Smith, J. K., Shanep, T. J., Haddon, M. E., Gunson, H. H., and Edwards, R. (1977), *Transfusion* **18,** 5.

The survival of Factor VIII in three grades of stored plasma which had been separated and frozen within 4 hours, 8 hours and 18 hours of blood donation. Results well presented.

—L.M.M.

Variability of Human Linkage Data. Rao, D. C., Keats, B. J. B., Morton, N. E., Yee, S., and Lew, R. (1978), *Am. J. Human Genetics.* **30,** 5.

The linkage data for 1,665 pairs of loci in 1,699 papers published up to the end of 1976. The purpose was to determine the frequency and cause of significant variability in construction of linkage maps. Good statistical

paper. Comprehensive list of Markers and to which Chromosome they have been assigned, presented.

—L.M.M.

Weak B Antigen in a Family. Boose, G. M., Issitt, C. H. and Issitt, P. D. (1977), *Transfusion* **18,** 5.

A family is described in which six members were of a weak subgroup of B. Presence of the subgroup in each individual was first indicated by lack of Anti B in the person's serum. Additional tests showed that the B subgroup red blood cells would absorb anti B and yield that antibody on elution. The blood of one of the family members with the weak B antigen was inadvertently transfused to a group O recipient with no ill effects.

L.M.M.

An Aseptic Fluid Transfer System for Blood and Blood Components. Mylise, B. A., Nakasako, Y. Y., Schott, R., Johnson, D., Berkman, R. M., and Clelano, E. L., (1978), *Transfusion* **18,** 5.

A system is described which can be adopted for use with all plastic collection systems. *In vivo* and *in vitro* studies showed that if blood was repeatedly passed through this system no cytological changes were evident and the *in vivo* survival of the Transfused material was not altered.

—L.M.M.

HLA Typing of Human Tumor Cell Lines Selection of Appropriate Typing Techniques. Kornbluth, J., Pllack, M. S., Fogh, J., Carey, T. and Dupont, B., (1978), *Transplantation Proceedings,* **10,** 4.

A systematic approach to the completed identification of HLA antigens on human tumour cell lines was undertaken. Appropriate adaptations of the standard complement-mediated lymphocyte monocytotoxicity test were developed for this purpose.

—L.M.M.

Microbiology

Evaluation of Routine Subcultures of Macroscopically Negative Blood Cultures for Detection of Anaerobes. Murray, R., and Sondag, Joan, E. (1978), *J. clin. Mic.* **8,** 427.

The routine subculture of macroscopically negative blood cultures did not significantly improve the detection of anaerobes. The authors tested 1688 blood cultures, 207 of which grew anaerobes. Most of these were detected macroscopically and the authors point out the cost involved in routinely subculturing all anaerobic blood cultures.

—S.G.

Factors Affecting Isolation and Identification of *Haemophilus vaginalis.* Bailey, R. K., Voss, J. L. and Smith, R. F. (1979), *J. clin. Mic.* **9,** 65.

This study compares the recovery of *H. vaginalis* on media incubated anaerobically and in carbon dioxide and media plated directly and after a delay of six hours. It also evaluates several methods of the identification of *H. vaginalis.*

—S.G.

Enzyme Immunoassays with Special Reference to ELISA Techniques. Volley, A., Bartlett, A. and Bidwell, D. E. (1978), *J. clin. Pathol.* **31,** 507.

This is a review of enzyme immunoassays, and in particular ELISA. Examples of the many applications of this technique are given.

—S.G.

Laboratory Identification and Epidemiology of Streptococcal Hospital Isolates. Hardy, Margaret A., Dalton, H. P. and Allison, M. J. (1978), *J. clin. Mic.* **8,** 534.

Three hundred and forty-three strains of streptococci were successfully identified by the scheme of Cowan and Steels Manual for the Identification of Medical Bacteria, and at the same time some of the biochemical tests and haemolysis on various types of blood agar were compared. The relationship of the strains to sex and age and their distribution in clinical specimens is given.

—S.G.

Preservation and Storage of Pathogenic Neisseria. Cody, R. M. (1978), *Health lab. Sci.* **15,** 206.

Neisseria gonorrhoeae and *Neisseria meningitidis* were kept alive for 12 months on chocolate agar slopes overlaid with oil, if these were kept at 30 C.

—S.G.

Serotypes and Antibiotic Susceptibilities of *Pseudomonas Aeruginosa* Isolates from Single Sputa of Cystic Fibrosis Patients. Seale, T. W.L Thirkill, H., Tarpay, Martha, Flux, M. and Rennert, P. M. (1979), *J. clin. Mic.* **9,** 72.

The authors tested multiple isolates of *Ps. aeruginosa* from single sputum specimens for serotype and antibiotic susceptibility. Their results show that cystic fibrosis patients may be infected with more than one strain of *Ps. aeruginosa*. As these strains may have different antibiotic sensitivities it is important to select multiple isolates from sputum samples for antibiotic sensitivity testing.

—S.G.

Significance of Appropriate Techniques and Media for Isolation and Identification of *Ureaplasma urealyticum* from Clinical Specimens. Kundsin, Ruth B., Parreno, A. and Poulin, Sharon (1978), *J. clin. Mic.* **8,** 445.

This paper suggests that the controversey over the association of *Ureaplasma urealyticum* with genitourinary tract infections and reproductive failure is related to the methods used to isolate the organism. They examine the types of specimens and some conditions of incubation and media used that might make a different to its isolation.

—S.G.

Erratum

An ABO Blood Group Anomaly
J. C. Coplestone (1979)

NZ J med Lab Technology **33,** 6

Last two photographs which were inadvertently omitted are reproduced here.

The answer to this case probably lies in an anomaly of T suppressor cells. Normally immunoglobulin is produced by plasma cells which are derived from lymphocytes. There may be a switch off in cases such as Chronic Lymphatic Leukaemia in the movement of cells from β resting cells to immunoglobulin producing plasma cells.

This control is under the auspices of T suppressor cells (Ts)—if Ts are activated excessively (e.g., if the Chronic Lymphatic Leukaemia is Chronic Lymphocyte (Ts) Leukaemia) then there will be supression of B cell activity, thus a suppresion of immunoglobulins.

SCI MED

Instrumentation & Technology
Science-Environment-Health

SCI-MED (N.Z.) LTD.

PRODUCT INFORMATION

SCI-MED N.Z. LTD. is a technical marketing organisation distributing to the science based customer in New Zealand and South Pacific Islands INSTRUMENTATION and TECHNOLOGY products

for – MED-LAB — Bio Chemical Instrumentation
BIO-MED — Physiological bioengineering
ANAL-IND — Chemical Analysis equipment
PHYS-LAB — Physics and Nuclear Instruments

Head Office:
P.O. BOX 321
DUNEDIN
Telephone 775-531

Regional Offices:
P.O. BOX 47309
AUCKLAND 3
Telephone 760-789

P.O. BOX 5221
CHRISTCHURCH
Telephone 799-050

P.O. BOX 6004
AWAPUNI
PALMERSTON
NORTH

MED - LAB

Bio-chemical Instrumentation for Prethology and Research Laboratories

DISCRETE ANALYSERS

I.L. MULTISTAT III micro-centrifugal analyser for drug screening, fluorescence and light scattering measurements. From "Instrumentation Laboratory". The well-proven "Giford" 300 series Med-Lab spectrophotometer which can be expanded into the system 3, 4, 5 or 3500 computer directed analyser and now used in more than 80 percent of New Zealand hospitals.

ELECTROLYTE ANALYSERS

From Instrumentation Laboratory Feature: IL443 or 643 Flame Photometers. IL446 Chloride/CO^2 analyser. IL919 Glucose Bun Creatinine. These can be expanded into the Multistat I, 4, 5 or 7 channel analyser by addition of the IL463 printer/sampler with interface to labs own data processor unit or I.L. D/P equipment

BLOOD GAS ANALYSERS

From Instrumentation Laboratory Feature: Micro-13 semi-automated 6 parameter system. To the IL813 automated analyser which can be part of the I.L. Blood Gas Laboratory when fitted with the IL282 co-oximeter. For THb, %O2, Hb, %Hb and Met. Hb, a new range of Blood Gas controls are available ex stock.

CONTINUOUS FLOW

$7,000 can purchase a complete two channel system. All modules are interfaceable with existing flow system from pumps, colorimeters, manifolds, samplers to eight channel Data Processors from CHEMLAB. All flow supplies available.

HAEMATOLOGY ANALYSERS

Fisher Scientific have introduced a new line of cell counters, automated coagulation timers, haemoglobinometers. I.E.C. — Plasma Analyser. Ortho Instruments introduce a new range of flow cytometry cell sorting equipment — cytofluorografs.

MICROBIOLOGICAL ANALYSERS

Rapid, automated, high sensitivity systems utilising Impedance measurements for detection of microbial activity. Data processing also available for large scale systems.

MED-LAB General

Please refer to chemical analysis section (ANAL-IND) for description of UV-VIS, AA, fluorescent spectrophotometers and HPLC-GC systems and other related equipment. Also refer (BIO-MED) for details of gamma counters, R.I.A, etc.

REAGENTS AND BIO-CHEMICALS

Fisher Scientific offer an extensive range of specialist reagents, buffers and now introduce the LEDERLE range of controls, standards for Bio-chem, Haematology, R.I.A. and Toxicology units. Gilford Worthington — Enzyme kits for your Gilford system ex stock New Zealand.

Sci-Med is now pleased to annouce its association with Hycel Inc., Manufacturers of Chemistry and Haematology Systems.

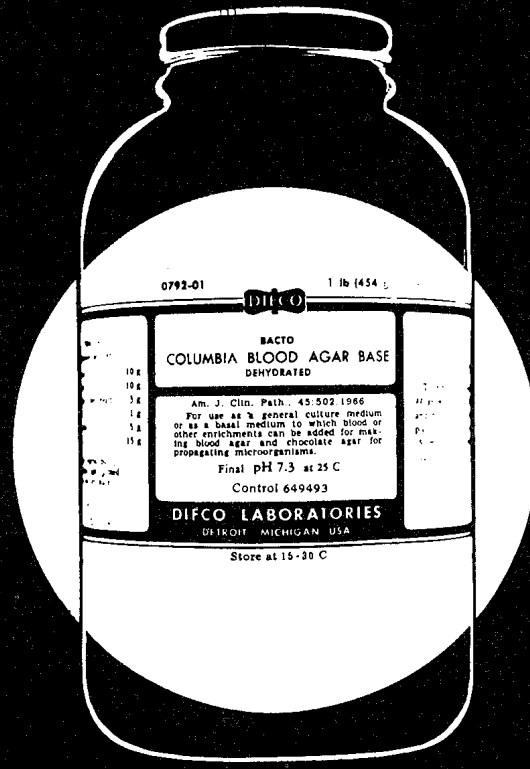
FOCUS ON PURITY AND QUALITY

DIFCO

DIFCO

World's most complete line used routinely wherever microbiological procedures are performed

Dehydrated media. Tissue culture media. Antigens and antisera. FA reagents. Stains. Dyes. Indicators. Serological reagents.



New Zealand's largest distributor and stockist: **GENERAL DIAGNOSTICS [NEW ZEALAND] LTD, P.O. BOX 7170, WELLINGTON.**

INDEX TO ADVERTISERS

Directions for Contributors

Original papers on topics related to medical laboratory science will be considered for publication. The original manuscript and one copy are required. Manuscripts should be typed on one side of the paper only, have 1 in margins and be double spaced. Give the author's name with initials or one first name if desired. Indicate the address of the laboratory where the work was carried out. Only use capitals where grammatically indicated and not for headings. Underline only where italics are required.

In general, papers other than reviews should consist of a short summary capable of standing alone as an abstract; an Introduction (outlining the problem and the proposed solution); Material and Methods; Results and Discussion.

Illustrations

Figures; cover graphs, photographs and drawings. The latter should be in black ink on stout paper. They should be about twice the size of the intended reproduction. Number consecutively with arabic numerals (1, 2, etc.) and identify on the back. Legends are typed on a separate sheet.

Tables are typed separately and numbered with roman numerals (I, II etc.).

Nomenclature and Units

Scientific names of micro-organisms should conform with Bergey's Manual of Determinative Bacteriology. The first time an organism is mentioned the full generic name should be given and underlined to indicate that it is to be printed in italics. Subsequently it may be abbreviated. Trivial or common names are printed in roman, e.g., staphylococci, and should not be underlined.

To conform with the Systemes Internationale D'Unites or SI units it is recommended that the following prefixes and abbreviations be employed.

Length: m, cm, mm, μm, nm.
Area: m^2, cm^2, mm^2, μm^2.
Volume: litre, ml, μl, nl, pl ('litre' in full avoids confusion with 'l')
Mass: kg, g, mg, μg, ng, pg
Mass concentrations: mol/litre, mmol/litre, μmol/litre, nmol/litre.
Temperature: Express as °C.
Time: s, min, h, d, a. The latter two symbols which stand for day and year respectively are best expressed in full to avoid confusion.
Density: kg/litre (relative density replaces 'specfic gravity')
Clearance: litre/s, ml/s

N.B.:

1. The symbol for a unit is unaltered in the plural and should not be followed by a full stop, e.g., 5 cm not 5 cm. nor 5 cms.

2. No space should be left between the symbol for a prefix and the unit. A space is left between the symbols in derived units,
   e.g., ms = millisecond
   m s = metre x second
   Where ambiguity could arise words should be written in full.

3. **Numbers.** The decimal is indicated by a full stop. Commas are not used to divide large numbers but a space is left after every third digit.
   A zero should precede numbers less than unity. Units which give a number between 0.1 and 1000 should be chosen when possible.

References should be listed alphabetically by author, at the end of the article and numbered in order. All authors' names must be listed with initials; year of publication in brackets; journal title abbreviated according to the World List of Scientific Periodicals. (In general nouns have capitals, adjectives do not and conjunctions are omitted. Previous journals may be consulted.) The title is underlined to indicate italics. This is followed by the volume number underlined with a wavy line to indicate bold type and finally the first page number.

Citations in the text are given the author's name using et al. if more than one, the year and the reference number as a superscript. Thus; Lowe *et al.* (1978)[1]

Proofs

When time permits, authors will be given the opportunity to correct proofs before publication, but not to insert new material. Proofs must be returned within three days of receipt.

Reprints

Reprints are available at cost price (minimum 50), from: The General Printing Department, Otago Daily Times, Box 181, Dunedin. An official order is required and must be received prior to publication. Orders may be submitted when returning the proofs.

CONTENTS