NOVEMBER, 1981

ISSN 0028-8349

THE NEW ZEALAND JOURNAL OF

medical laboratory technology

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

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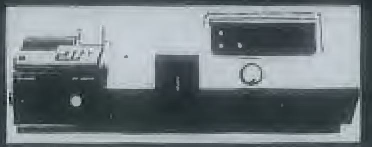

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THE NEW ZEALAND JOURNAL OF

Medical Laboratory Technology

Vol. 35, No. 3 ISSN 0028-8349 November 1981

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Identification of Human Chromosomes Using G-banding Techniques

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Received for publication February 1981

Summary
This paper describes techniques for the production of G-bands in human chromosomes from cultures of blood, marrow, fibroblasts and amniotic cell cultures. The bands are produced by treatment of chromosomes in a hot alkali solution, followed by trypsinisation with subsequent staining by Giemsa or Leishman.

Introduction
Numerous chromosome banding procedures have been developed over the past decade. The staining patterns produced fall into four main groups (Paris Conference 1971): "Q-bands", fluorescent banding with quinicrine dyes; "G-bands", produced by Giemsa staining following various pretreatments and corresponding in general to Q-bands, i.e. deeply stained G-bands are brightly fluorescent in Q-band preparations; reverse (R) bands, in which Giemsa stain is taken up in quinicrine-negative, rather than quinicrine-positive, regions; and C-(Centromere-) banding, which in human chromosomes is seen on the centromeres, the secondary constrictions of chromosomes, 1, 9, 16 and the distal part of the long arm of the Y chromosome (Figure 1). These advances in the identification of human chromosomes made it necessary to introduce a standard system of nomenclature to describe individual chromosomes and chromosome regions. This nomenclature, based on the Q-, G- and R- banding techniques, was established at the Fourth International Congress of Human Genetics in Paris, 1971.

Figure 1. C-bands in the normal male complement 46, XY.

The fundamental basis or mechanism of chromosome banding is still not clear, except to say that the dyes involved are essentially low molecular weight probes which are sensitive to different types of chromatin in the human genome.

Since the beginning of the last decade, when Caspersson *et al.* (1970)[1] first made it possible to identify every chromosome using quinicrine dyes activated with U.V. light, a simpler, less complicated technique, with no need for expensive fluorescence microscopes was urgently needed to be incorporated into the everyday routine of the Cytogenetic Laboratory.

This prompted a spate of papers in 1971 and 1972 introducing the now commonly used G-banding methodologies, so-called because the bands which have near identical distribution as Q-bands (Figure 2) are produced by staining with Giemsa (Figure 3). Sumner *et al.* (1971)[7]; Drets and Shaw (1971)[2]; Patil *et al.* (1971)[4]; Schnedl (1971a, b)[5]; Seabright (1971)[6]; Wang and Fedorof (1972)[9]; Kato and Yosida (1972)[3]; Utakoji (1972)[8].

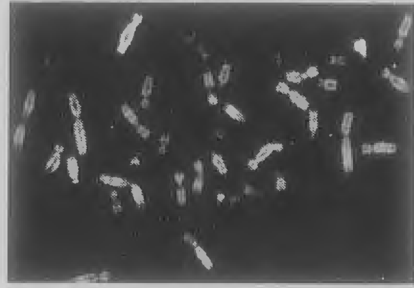

Figure 2. Partial male karyotype showing Q-bands. Note brightly-fluorescent distal long arm of the Y chromosome.

The results of the different procedures are very similar to one another and, over a period of time, have been modified so that now no two laboratories carry out the same "recipe" in its production of G-banded karyotypes.

The object of this paper is to give details of the G-banding methods used in our own laboratory, so that the reader may, hopefully, be able to

reproduce similar results on metaphases from blood, marrow, fibroblast and amniotic cell cultures.

The methods we use are based on a combination of two separate procedures developed by Seabright (1971)[6] and Sumner *et al.* (1971)[7].

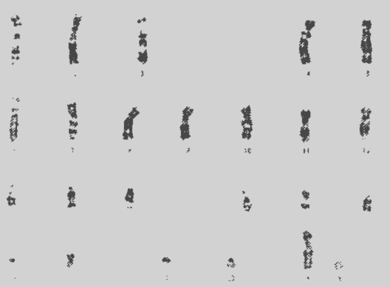

Figure 3. G-bands seen in the human genome.

Materials and Methods

Methods for G-banding of metaphases from 48 and 72 hour human blood cultures.

Solutions and Reagents

1. *Working Solution of 2 × SSC*

Sodium chloride	17.53 g
Trisodium citrate	8.82 g
Deionised or Dist. H_2O	1000 mls

2. *Trypsin bactotrypsin* (Difco Cat. No. 0153)
   10 ml of sterile Isotonic saline is added to a phial of bactotrypsin. 1 ml of this solution is diluted with 9 ml of isotonic saline for a working solution.

3. *Giemsa R66 stain*
   5 ml of Giemsa R66 made up to 20 ml with pH 6.8 Sorensen's phosphate buffer.

4. *Leishman Stain*
   5 ml of Leishman stain made up to 20 ml with pH 6.8 Sorensen's phosphate buffer.

Procedure

1. Human blood cultures are made in the normal way. The metaphases are harvested after 48 or 72 hours, treated with a hypotonic solution, fixed in three changes of methanol/acetic acid (three parts to one part), spread and air-dried.
2. Slides are incubated for two hours at 60°C in 2 × SSC solution.
3. Slides are then rinsed in isotonic saline, Dist. H_2O and put through graded alcohols 70%-100% and allowed to dry in air.
4. Slides are then treated in working trypsin solution for 20-30 seconds.
5. Slides are then rinsed × 3 in isotonic saline.
6. Slides are then stained in solution of Giemsa or Leishman for 4 to 6 minutes, washed rapidly in pH 6.8 buffer and blotted dry. Examine under microscope.

Notes on Procedure

1. Put through control slides of same age to test time in trypsin solution. In this laboratory, best results are obtained on slides three-five days old.
2. The optimal results are obtained in metaphases of good morphology. In contracted chromosomes the finer bands tend to merge together so that only the main bands show.
3. Trypsin treatment increases the size of the chromosomes by swelling the chromatids, which appear to be joined together.
4. Over-trypsinisation will result in complete denaturation of chromatin, resulting in elimination of all bands.
5. Staining times in Giemsa and Leishman will vary from batch to batch. Over-staining will obscure bands.
6. In step 3 of Procedures, rinsing slides in graded alcohol solutions can be omitted, washing only in isotonic saline before proceeding to step 4.
7. Flame-dried preparations are more resistant to trypsinisation than air-dried preparations. We recommend air-dried preparations for all banding techniques.
8. All stages other than staining are carried out using Coplin jars.

Method for G-banding of Metaphases from Indirect and Direct Marrow, Fibroblast and Amniotic Cell Cultures.

Solutions and Reagents

The same as for blood cultures, except that a weaker solution of trypsin is used, as follows: 0.25 ml of bactotrypsin is diluted to 20 ml with isotonic saline.

Procedure

1. Incubate slides in 2 × SSC for one hour at 40°C. Then same procedure as for blood culture metaphases.
2. Treat in weak solution of trypsin for 10-20 seconds. Then same procedure as for blood culture metaphases.

Notes on Procedure

1. As with blood cultures, best results are obtained with metaphases of good morphology.
2. Length of time in trypsin is usually less than that required by blood culture metaphases. Again, put through a control slide same age as test slide.

Method for G-banding of Pre-stained Marrow, Blood, Fibroblast and Amniotic Cell Preparations.

There may arise occasions when it could be necessary to destain preparations which have been prepared in the non-banded conventional style. We find the following method very reliable, and often use it on marrow preparations when metaphases are scanty (Figures 4a, b, c).

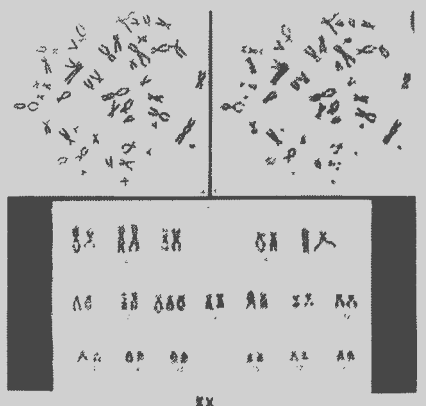

Figure 4. (a) Metaphase from marrow culture stained by conventional means.
(b) G-band pattern of (a) after destaining.
(c) Cut-out karyotypes of (b) showing trisomy for chromosome 8. 47, XX, + 8.

Solutions and Reagents

The same as for G-banding of Indirect and Direct marrow cultures.

Procedure

1. Remove immersion oil in xylene—three minutes.
2. Remove xylene by washing in methanol.
3. Destain in methanol/acetic acid fixative solution—approximately five minutes.
4. Rinse in methanol and allow to air-dry.
5. Incubate in 2 × SSC solution at 40°C for one hour.
6. Rinse in isotonic saline.
7. Treat slides in weak solution of trypsin—20-30 seconds.
8. Rinse in isotonic saline.
9. Stain in Giemsa or Leishman for four-six minutes. Wash rapidly with pH 6.8 buffer, blot-dry, and examine under microscope.

Notes on Procedure

1. Slides that have been previously stained and mounted in DPX will not produce good banding with the above technique.
2. If necessary, the whole procedure can be repeated lengthening time in trypsin solution if cells do not show evidence of banding.

Results and Discussion

The techniques given in the preceding schedules are intended to be carried out on conventionally-processed chromosome preparations. One may have to experiment slightly away from the rigidity of the methods given to suit prevailing conditions in a particular laboratory. Nevertheless, the techniques given have proven not just successful in the hands of trained staff in our own laboratory, but likewise in the hands of trainee students.

The key to all banding procedures is good culture preparations. Bands are produced more clearly in chromosomes which are still elongated, and in which chromatids are still lying adjacent to each other.

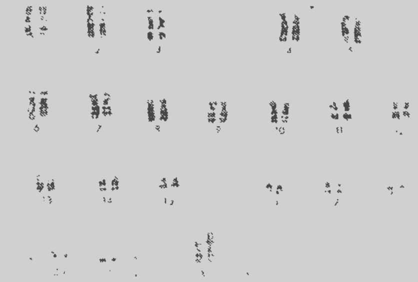

Figure 5. G-banded karyotype of Turner's syndrome showing a long arm isochromosome of the late replicating X chromosome. 46, X, i (Xq).

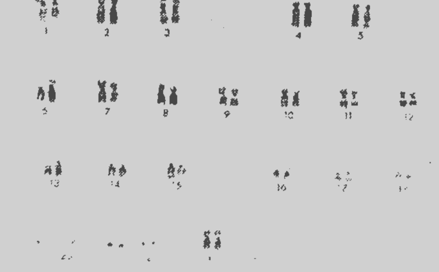

Figure 6. G-banded karyotype showing short arm deletion of one of the number 18 homologues. 46, XX, 18p.

The introduction of banding techniques has seen a spectacular advancement in the diagnosis of clinical disorders (Figure 5). It is now commonplace to see reports of monosomies and trisomies being identified on just segments of chromosomes (Figure 6). A continuing value has also been seen in their application to cancer cytogenetics and human gene mapping. No modern cytogenetic laboratory can be said to be offering a service without endeavouring to incorporate into its routine good, reliable G-banding techniques.

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A Modified Cresolphthalein Complexone Method for Manual Calcium Estimation

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Received for publication September 1981

Summary

A manual Cresolphthalein Complexone (CPC) calcium method in which no toxic reagents are involved is presented. It uses an acid CPC reagent, lacking the usual Dimethyl Sulphoxide (DMSO) stabilising reagent, and Sodium meta-periodate stabilised 2-Amino-2-methyl-1-propanol (AMP) as the alkalising agent. Calcium is complexed with CPC in acid solution. At alkaline pH the Ca^{2+}/CPC complex is highly coloured. Magnesium interference is eleminated by preferential chelation with 8-Hydroxyquinoline. A description of optimal reagent concentrations and other procedural parameters is given.

Introduction

Since the original use of CPC for calcium analysis by Kessler *et al* (1964)[2] many publications have been published, most of these for automated use. Early methods utilised Diethylamine (DEA) as the alkalising agent at such a concentration to achieve a final pH of 13. At this pH Magnesium interference was reduced but CPC acted as an acid/base indicator resulting in colour production even in the absence of calcium. With Gitelman's (1967)[1] introduction of 8-Hydroxyquinoline to eliminate magnesium interference, the need to maintain the high pH no longer existed. The problem of the high absorbance blank was remedied when Moorehead *et al* (1974)[3] replaced the toxic DEA with the nontoxic aminoalcohol, 2-Amino-2-methyl-1-propanol, resulting in a final pH of approximately 10, at which pH the uncomplexed CPC is virtually colourless.

A number of manual CPC/DEA methods have been published but few manual methods utilising AMP as alkalising agent. The CPC/DEA method in use in this Laboratory[4] exhibited the usual high blank absorbance and a concurrent nonlinearity. See figure 1. An attempt was made to reduce the blank absorbance to negligible levels, to improve linearity and at the same time to eleminate the toxic agents. The method presented appears to meet these aims.

Materials and Methods

All reagents should be prepared in acid washed glassware using deionised or double distilled water.

Cresolphthalein complexone reagent

o-Cresolphthalein complexone	0.015g
8-Hydroxyquinoline	1.25g
Hydrochloric acid (concentrated)	1.5ml

With care, dissolve the reagents in the acid. A few millilitres of water may be added. When solution is complete make up to 500ml with water. Store at room temperature.

Alkalising reagent

Sodium meta-periodate	0.75g

Dissolve in approximately 100ml of water. Add 75ml 2-Amino-2-methyl-1-propanol AR. Make up to 500ml with water. Store at room temperature. Reagent shows negligible deterioration during the first week with blank absorbances of approximately 0.080, then blank absorbance slowly increases to reach approximately 0.140 after four weeks.

Standard solution

Stock (25.0mmol 1). Dissolve 2.502g of dry anhydrous Calcium Carbonate AR in the smallest possible volume of concentrated Hydrochloric Acid. Take great care to avoid spattering. Make up to 1.00 litre. Working (2.50mmol/l). Dilute 10.0ml of stock standard to 100ml with water.

Procedure

Dispensers mysteriously accelerate the rate of deterioration of the alkalising reagent. Nichyro and Oxford dispensers display this property. Other brands have not been tried. After some use the contaminant elutes from the dispensers.

1. To separately labelled disposable plastic test tubes, add 50μl of serum or standard.
2. To each tube plus another labelled blank, add 2.5ml Cresolphthalein complexone reagent. Mix.
3. To all tubes add 2.5ml of alkalising reagent. Mix thoroughly.
4. Read absorbance of the test and standard against the blank in a spectrophotometer set at 575nm. Colour is stable for at least 20 minutes. (See note later regarding temperature).

Calculation

$$\frac{\text{Absorbance of test} \times 2.5}{\text{Absorbance of standard}} = \text{mmol/l calcium}$$

Discussion

Initial experiments were carried out by simply substituting the DEA reagent with various aqueous dilutions of AMP, however the AMP rapidly deteriorated with increasing blank absorbances. The solution was able to be stabilised with lg/l potassium cyanide (KCN) however it was decided to attempt to replace the KCN with a nontoxic reagent. A number of chemicals with oxido-reductive properties were tested and after some experimentation it was found that

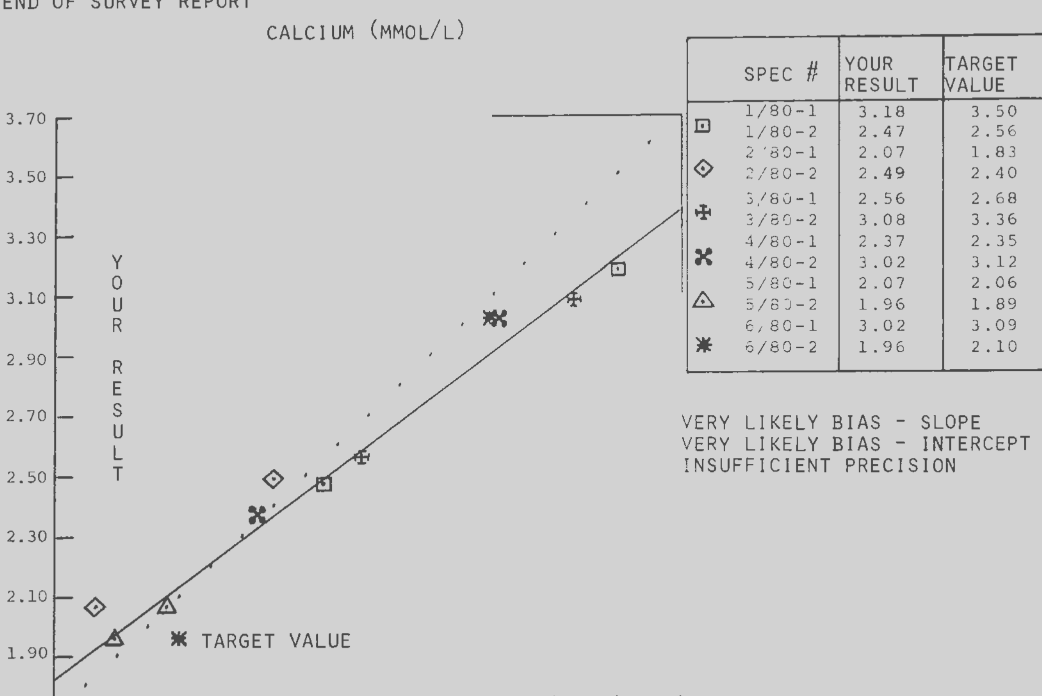

Figure 1—MRC Survey results showing nonlinearity of existing CPC/DEA method.

sodium meta-periodate was an adequate substitute.

Autoanalyser CPC reagents[3] did not contain dimethyl sulphoxide and it was decided to attempt to produce a stable CPC reagent without the offensive DMSO. By dissolving the CPC and 8-hydroxyquinoline in acid prior to dilution to volume, this object was achieved.

A series of experiments were then conducted to optimise the concentrations of each of the reagent consitutents and procedural parameters.

Cresophthalein complexone. A series of CPC reagents were prepared with concentrations of CPC between 16 and 160μmol/l. It was found that blank absorbance increased with increasing CPC concentration. The minimum concentration to achieve linearity to 5mmol/l Ca^{2+} was found to be 47μmol/L. See figure 2. The linear range can be greatly extended without markedly increasing the blank absorbance, by increasing the CPC concentration.

Hydrochloric Acid. Optimal sensitivity with minimal blank absorbance was achieved with a concentration of 3.0ml concentrated hydrochloric acid per litre of reagent. This volume also provided a convenient volume in which to dissolve the CPC and 8-hydroxyquinoline.

8-Hydroxyquinoline. The concentration of this was left unchanged from the original method.

2-Amino-2-methyl-l-propanol. Concentrations between 10g/l and 250g/l AMP were tested with the final CPC reagent. A final concentration of 150g/l was chosen but concentrations between 120 and 200g/l have little effect on blank absorbance or sensitivity.

Sodium meta-periodate. Concentrations between 0.6g/l and 4.5g/l were tested for their ability to stabilise the AMP reagent. 1.5g/l was found to provide adequate stabilisation without adversely affecting the blank absorbance.

Temperature dependence. In an effort to eliminate an apparent colour instability problem, the effect of temperature on absorbance was investigated, resulting in the discovery that CPC calcium methods are particularly sensitive to temperature changes. In spectrophotometers with non-temperature controlled cuvettes this results in apparent instability while the solution slowly adjusts to the temperature of the cuvette. Naturally the degree of "instability" depends upon the difference between the cuvette temperature and ambient temperature. This fact probably accounts for the instability that has plagued these methods. If absorbance is measured in a thermoregulated cuvette the stability of the final colour is excellent, and remains so for at least 20 minutes. Absorbance increases with decreasing temperature. There is a concurrent increase in blank absorbance. This property is not peculiar to the proposed method. See figure 3.

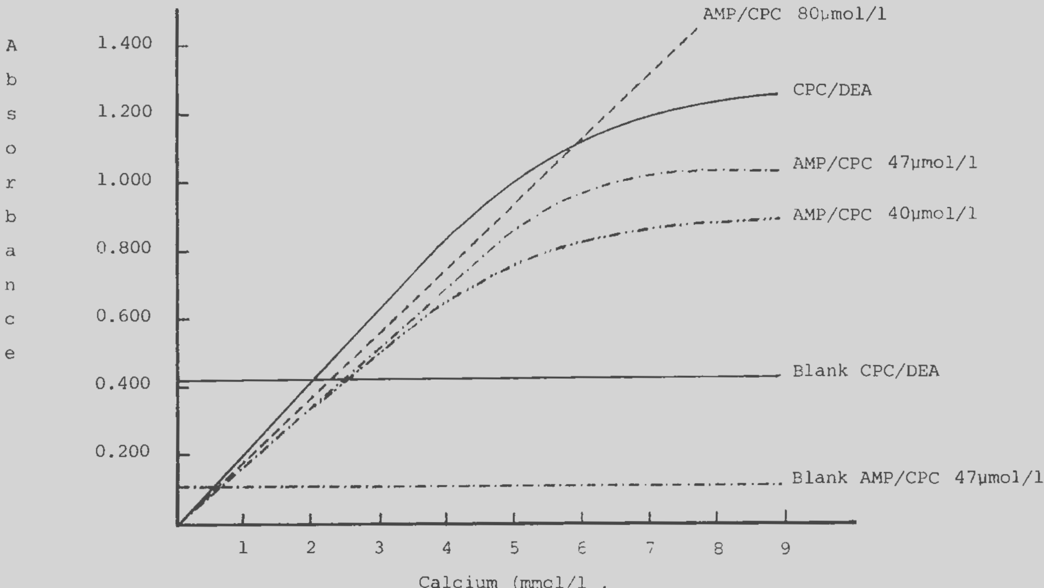

Figure 2—Effect of CPC concentration on linearity of CPC/AMP method.

Absorbance
1.000
0.800
0.600
0.400
0.200
DEA Test (2.5mmol/l)
AMP Test (2.5mmol/l)
DEA Blank
AMP Blank
26 28 30 32 34 36 38
Temperature °C.

Figure 3—Effect of temperature on Absorbance (versus water blank) of CPC/AMP and CPC/DEA calcium analyses.

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Table I—Effect of added Magnesium on calcium analysis.

	Mg^{2+} concentration	Mean absorbance
Aqueous standard	2.0mmol/l	0.006
	4.0mmol/l	0.000
	6.0mmol/l	0.000
	8.0mmol/l	0.001
serum	1.6mmol/l	0.686
	3.6mmol/l	0.690
	5.6mmol/l	0.686
	7.6mmol/l	0.685
	9.6mmol/l	0.686

Validation of method

With final "optimised" reagents the following checks were made on the method:

Blank absorbance. Under the specified conditions of the test the reagent blank is approximately 0.080.

Linearity. The method was checked for linearity using aqueous dilutions of a commercial standard, with concentrations between 1.25 and 12.5mmol/l. (Titrisol for serum analysis. E. Merck, Darmstadt West Germany). The existing CPC/DEA method was tested in parallel. Refer figure 2.

Magnesium interference. The method was checked for magnesium interference by analysing pairs of pure magnesium standards and by addition of magnesium to serum. Refer Table I.

Accuracy. A double blind study was carried out to compare the results from the proposed method with those obtained by Atomic Absorption Spectrophotometry. (Atomic Absorption work was carried out by the Trace Metal Laboratory at Dunedin Hospital.

Linear regression analysis. The following linear regression equation was obtained:

$y = 0.982x = 0.027$ x = A.A. y = CPC/AMP

Correlation coefficient = 0.9904

Compared with atomic absorption results, the proposed method shows a bias of −0.07mmol/l. Although this is highly significant statistically, subsequent work suggests that this bias was an aberration of the particular series of analyses, namely:

The mean of eight analyses of an elevated control sera (Q-PAK-Chemistry Control Serum II, Hyland Diagnostics, Division of Travenol Laboratories Inc. U.S.A.), target value 3.15mmol/l was 3.18mmol/l.

In routine use a normal control sera (Precinom, Boehringer Mannheim. W. Germany), target value 2.14mmol/l gave the following parameters:

n = 32, mean 2.13mmol/l, S.D. 0.034mmol/l.

Table II—Comparison of results by proposed method and Atomic Absorption Spectrophotometry.

Serum Number	Ca^{2+} mmol/l CPC/AMP	Ca^{2+} Atomic A.
1	2.52	2.47
2	2.33	2.40
3	2.20	2.21
4	2.32	2.39
5	2.30	2.33
6	2.38	2.50
7	2.07	2.12
8	2.36	2.42
9	2.19	2.30
10	2.20	2.28
11	2.36	2.50
12	1.85	1.95
13	2.12	2.21
14	2.97	3.07
15	3.22	3.30
Mean	2.36mmol/l	2.43mmol/l

Participation in the Wellcome Group Quality Control Programme gave the following results for the means of results of the first 8 samples in the current series:

All method mean	:	2.631mmol/l
Method Group mean	:	2.645mmol/l
Method Mean	:	2.672mmol/l
Proposed method	:	2.657mmol/l

Precision. Within batch reproducibility. Data relates to 51 serum samples analysed in duplicate. Standard deviation was determined using the formula

$$SD = \sum \frac{d^2}{2n}$$

Where d is the difference between the duplicate pairs and n is the number sampled. The standard deviation was 0.029mmol/l, mean 2.40mmol/l giving a coefficient of variation of 0.012.

Between batch reproducibility as assessed from our high and normal unassayed control sera gave the following results:

High	Normal
n = 22	n = 22
mean = 3.0mmol/l	mean = 2.27mmol/l
S.D. = 0.069	S.D. = 0.054
C.V. = 0.023	C.V. = 0.024

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Toxocara Infection Resulting in the Formation of an Anti A in a Group A_1 Individual

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Received for publication 17 February 1981

Summary

The production of blood group substance reacting antibodies following bacterial, or parasitic infection is well documented, Marsh (1970)[6], Oliver-Gonzalez *et al* (1943)[7]. This paper describes a case of Toxocara infection in a patient that prompted the production of an antibody that reacted with their own, and all Group A cells with which it was tested.

Case History

An 18 month old Polynesian child presented to Out Patients with a persistent cough, and fever. The child appeared pale, and irritable. Initial haematological findings were, Total white cell count 54.7 x 10^9/litre, Haemoglobin 61g/litre, Packed Cell Volume 0.27, MCHC 230, Reticulocyte Count 0.044. A blood film examination revealed moderate anisocytosis and hypochromia, some microcytes, polychromasia, and a marked eosinophilia, absolute values of 31.18 x 10^9/litre being obtained. The child was admitted for further observation. Bone marrow obtained from the iliac crest contained a predominance of mature, and developing eosinophils. Erythropoiesis was normoblastic. Findings suggested a parasitic infection.

The patient exhibited mild hepatomegaly, and bronchial involvement. No occular involvement was detected, Ashton (1960)[1], Duguid (1961)[2]. Serum proteins were increased, the gamma globulin level markedly so, Heiner *et al* (1956)[3]. A history of pica was determined. Initial treatment of the anaemia was begun by requesting one unit of semi-packed red cells. The patient typed as Group A Rh D positive. Incompatibilities were detected by saline, and enzyme technique, marked rouleaux also being present, the patient having increased serum proteins. Further tests were undertaken to determine the incompatibility.

Methods:

The patient's serum was tested against a panel of 10 Group O cells fully typed for common inherited antigens, and also $2A_1$, $2A_2$, and Group O cord red cells by the use of Low Ionic Strength Saline (L.I.S.S.) at room temperature, enzyme 37°C, and indirect Coombs Technique. Equal volumes of patient's serum, and 3% L.I.S.S. suspended cells were used. A 1% Papain solution was used by a layering technique. Incubation times were 10, 15, and 10 minutes respectively. The saline room temperature tubes were read microscopically after centrifugation at 1000 r.p.m. for 10 seconds. Rouleaux was dispersed by the the addition of saline. The enzyme was examined macroscopically over a concave mirror after centrifugation at 1000 r.p.m. for 15 seconds.

Tubes for the L.I.S.S. Indirect Coombs method were after incubation, washed four times in saline. The cell button being resuspended, and 1 drop of a Broad Spectrum Anti-Human Serum being added. The tubes were then centrifuged at 1000 r.p.m. for 10 seconds, and examined macroscopically over a concave mirror. All negative tests had 1 drop of 3% Coombs control cells added, centrifuged, and were then re-examined.

Agglutination was observed at room temperature with Group A cells only, both A_1, and A_2 cells giving equally positive reactions. Group O cells including the cord cells were negative. A subtyping using the lectin from *Dolichos biflorus* showed the patient to be Group A_1. A Direct Coombs test was negative with broad spectrum, and monospecific Anti-Human Serum.

The patient's serum was incubated for 1 hour at 4°C, and room temperature against 3-5% physiological saline suspensions of 10 Group A_1, and 10 Group A_2 cells, and two Group A cord cells. All tubes were examined microscopically. Agglutination occurred with all cells, slightly weaker reactions being noted with the cord samples. Titration studies were performed using saline suspended Group A_1, and Group A_2 cells at 4°C, 15°C, and room temperature. Incubation was for one hour with physiological saline being used as the serum diluent. All tubes were read microscopically, the results obtained are shown on Table I.

TABLE 1

A1 CELLS	**Titre**	**Score**
Ambient Temp.	2	8
15°C	16	36
4°C	32	53
A2 CELLS	**Titre**	**Score**
Ambient	2	8
15°C	8	33
4°C	32	46

It has been shown that increased titres of Anti A and Anti B have been found in patients suffering from Visceral Larval Migrans Syndrome, Heiner (1962)[4], Huntly *et al* (1965)[5]. Titration on admission of this patient's natural Anti B at room temperature using a 3-5% saline suspension of Group B cells, with 1 hour incubation, was greater than 1:5000. Dilution of the patient's serum were made in physiological saline. The tubes were examined microscopically. No haemolysin was demonstrated at any time. Precipitin techniques were attempted using blood group substance, without success. Faecal specimens failed to yield any ova, and negative occult blood tests were obtained. Serological examination was positive for Toxocara infestation by Elisa technique, a titre of greater than 1:256 being obtained.

Treatment

The anaemia was treated by the transfusion of a unit of packed Group O Rh D positive red cells. No adverse problems were encountered. Administration of Thiabendazole twice daily for five days was commenced. The child was discharged from hospital four days later, with a total white cell count of 26.3 x 10^9/litre, haemoglobin 126g/litre, and an absolute eosinophil count of 18.66 x 10^9/litre. A high eosinophil count was still demonstrable five months later and Diethylcarbamazene was administered. A gradual decrease in eosinophil count over the following months is expected.

Discussion

Visceral larva migrans syndrome in humans is well documented, Woodruff (1975)[9]. It has been shown to occur with a greater incidence in children from 18 months to four years. The definitive diagnosis of Toxocara infestation can be made only by the isolation of ova from the stools. This can however prove difficult. Presumptive diagnosis in cases exhibiting the following symptoms has been recommended, Shrand (1964)[8].

Symptoms:
1. Most common age group 1—4 years; 2. Anaemia Haemoglobin values < 90g/litre; 3. Eosinophilia $>30\%$; 4. Hepatomegaly; 5. Pica; 6. Bronchial involvement; 7. Occular involvement; 8. Skin lesions.

Serological aids to diagnosis are:
(a) Toxocara antibody titres >1:32; (b) High titre anti A or anti B >1:520; (c) Increased total protein, markedly increased γ globulin.

Conclusion

The sanitary conditions of this family were found to be grossly inadequate. The family possessed no flush toilet, a pit in the yard area being used. No hot or cold water to the kitchen sink, no shower, bath, or hand basin were available. A kitten was owned by the family, and the child played with this animal in the yard area. Serological tests performed three months, and five months later failed to demonstrate the presence of the abnormal A agglutination previously detected. Repeated titrations of the natural anti B of 1:5000, and 1:2048 respectively were obtained at these times. Methods used were as previously described.

It would appear that following the removal of the infective agent, by treatment with Thiabendazole, and Diethylcarbamazene, the antigenic stimulus for the production of the blood group antigen reacting antibody was similarly removed by relocation of the family into a more sanitary environment, and removal of the possible source of Toxocara ova, the kitten, it is hoped to prevent a recurrence of this problem.

Acknowledgement

I wish to thank Mrs D. Williamson for her assistance in locating references and Mrs K. Battin for preparing this article for print.

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Characterisation of seven type cultures of Anaerobic Bacteria by Gas Liquid Chromatography using methods and keys from the Center for Disease Control (Atlanta) Georgia and the Virginia Polytechnic Institute. A Comparison

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Received for publication August 1981

Summary

Two methods of gas liquid chromatography (GLC), were used to determine the fatty acids and alcohols, produced by seven type cultures of anaerobic bacteria and possible identifications were worked out using key charts.

Results indicated that the method from the Center for Disease Control (CDC) was superior to the method from the Virginia Polytechnic Institute (VPI). The limitations and usefulness of the techniques are discussed.

Introduction

The use of gas liquid chromatography (GLC) in clinical laboratories has not yet been fully clarified and a standard method is not available for the characterisation of anaerobic bacteria.

In this study two methods of GLC. one from the Anaerobe Laboratory Manual of the Virginia Polythechnic Institute (VPI) (Holdeman *et al*, 1977)[1] and one from the Center for Disease Control (CDC) anaerobe laboratory (Lombard, 1977)[2] were used to obtain GLC profiles of the alcohols and fatty acids produced by seven type cultures. The results are compared and used to illustrate the limitations of GLC for characterising anaerobes in the clinical laboratory.

Materials and Methods

The following type cultures were supplied to Green Lane Hospital (GLH) by National Health Institute (NHI), Wellington; *Bacteroides fragilis* NCTC 8560, *Bacteroides vulgatus* Wadsworth 1887, *Bacteroides asaccharolyticus* NCTC 9337, *Bacteroides capillosus* VPI 10874, *Clostridium bifermentans* NCTC 1340, *Clostridium difficile* NCTC 11223 and *Clostridium histolyticum* CDC 1942.

Cultures to be used with VPI key charts were grown in peptone yeast glucose broth (PYG) made with 1% yeast extract, 1% glucose, 0.5% peptone, 0.5% trypticase, 0.5mg/100ml haemin and 100 ug/100ml vitamin K_1, as described by VPI. The medium for the CDC method was also PYG broth, but it contained no haemin or vitamin K_1, and 1% peptone replaced 0.5% peptone and 0.5% trypticase.

After 48 hours or when good growth had been obtained extractions were made for alcohols and volatile fatty acids, and, methylation and extraction for non-volatile fatty acids according to the VPI and CDC laboratory methods.

A Varian 3700 Gas Chromatograph equipped with a flame ionization detector was used for analysis.

The GLC column contained 10% SP-1000/1% H_3PO_4 on 100/120 Chromosorb WAW, the carrier gas was nitrogen and temperature programming was used. For detection of alcohols and volatile fatty acids oven temperature was at 80°C for injection, remained 80°C for one minute and was then increased 30°C/min to 160°C.

For detection of non-volatile fatty acids oven temperature was 130°C at injection and changed to optimum of 160°C in one minute. Standard solutions of alcohols and fatty acids as recommended by VPI and CDC were used with each batch of specimens, and internal standards, heptanoic, valeric or methyl malonic acids were added according to specimen.

Results were expressed as identity of each substance and major or minor quantity of each fatty acid detected by VPI method. CDC results were expressed as identity of fatty acid. All results were compared with their respective key charts. GLC profiles of type species found in the Additional Information sections of the VPI Anaerobe Laboratory Manual were also compared with VPI key charts.

Results

The table shows metabolic products from PYG detected by GLC. Metabolic products produced by the seven type cultures from NHI and products produced by type species as given in VPI Anaerobe Laboratory Manual are compared with VPI and CDC key patterns.

Bacteroides fragilis

Chromatograph patterns produced by NHI type species agreed with both VPI and CDC key charts. VPI key offered 13 possible identifications, *B. fragilis, B. vulgatus, B. thetaiotaomicron, B. distasonis, B. ovatus, B. melaninogenicus ss melaninogenicus, B. uniformis, B. eggerthii, B. bivius, B. succinogenes, B. ochraceus, B. ruminicola ss ruminicola* and *B. ruminicola ss brevis.* CDC key gave five of the above possibilities in its identification, the former *B. fragilis* group.

The type species used as an example in VPI Anaerobe Manual produced more propionic acid than would be expected from the VPI key chromatograph chart.

Bacteroides vulgatus.

B. vulgatus and *B. fragilis* have identical key chromatographic patterns and results obtained at GLH were in agreement with these.

Bacteroides asaccharolyticus

The type species tested at GLH did not produce sufficient butyric or acetic acid to fit the VPI identification key for *B. asaccharolyticus.* The same micro-organism gave correct specific identification using the CDC key.

The VPI type species differed from VPI identification key as to the quantity of butyric acid produced.

Bacteroides capillosus

Results from GLH did not fit with VPI or CDC keys for *B. capillosus* or any other *Bacteroides* species. Quantities of acids produced were too large for VPI and number of acids produced were too many for CDC key.

The type species chosen by VPI produced major quantities of acetic and succinic acid at variance with the VPI identification key.

Clostridium bifermentans

GLH chromatographic patterns fitted both

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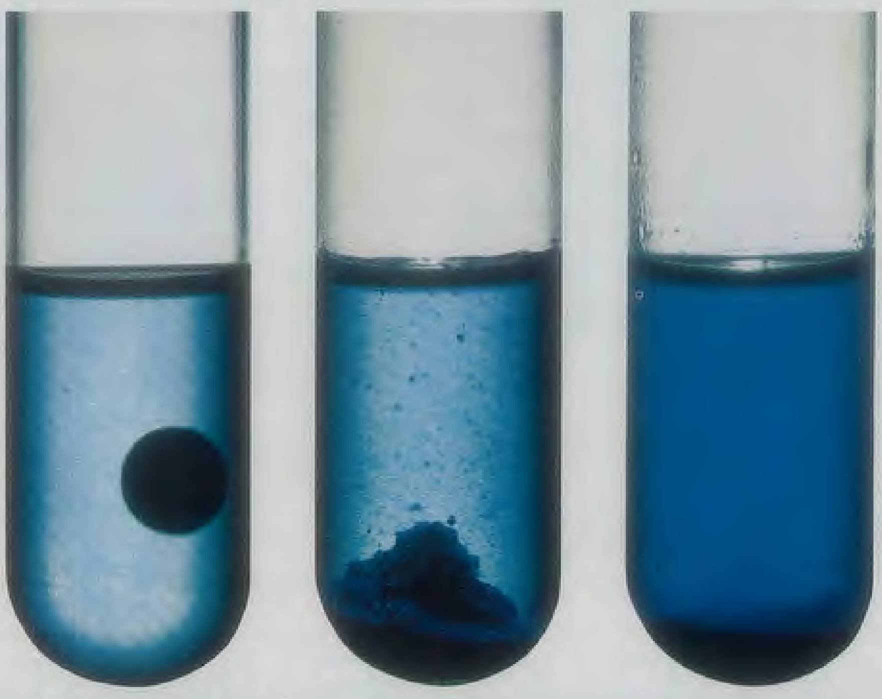
R_1 N N CH_2OH CH_2OH CH_2O N R_2 O O O O OH O OH O OH OH OH OH n

Dye bound covalently to amylose instead of starch as substrate



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identification keys. Identification was specific by VPI key, but CDC key gave two possibilities *C. bifermentans* and *C. sordellii.*

The VPI type species produced minor instead of major quantities of acetic acid as stipulated in the VPI identification key.

Clostridium difficile

GLH chromatographic patterns varied from VPI identification key in production of ethanol and butanol and quantity of butyric acid. No other identification was possible. When CDC key was used six identification possibilities were obtained: *C. difficile, C. botulinum* proteolytic types A, B and F, *C. sporogenes* and *C. subterminale.*

VPI types species differed from VPI identification key in production of ethanol and butanol.

Clostridium histolyticum

For this micro-organism identification keys and type species were all in agreement. Alternative identifications offered by VPI were *C. histolyticum, C. limosum, C. clostridiiforme, C. leptum, C. aminovalericum* and *C. bifermentans.* For CDC alternatives were *C. bifermentans, C. histolyticum, C. limosum* and *C. sordellii.*

Discussion

Three of the 7 type cultures gave GLC profiles that did not fit the VPI identification key and one type culture gave a GLC profile that did not fit the CDC identification key. An unexpected finding was that profiles from the Additional Information sections of the VPI Anaerobe Laboratory Manual were correct for only two of the seven anaerobes using the VPI key.

The failure of the VPI key on three occasions was due to its dependence on the quantitation of fatty acids. If precise quantitation was relaxed correct fatty acid

KEY TO TABLE

Volatile Acids	VPI Minor Quantity < 1 milliequivalent/100ml	VPI Major Quantity > 1 milliequivalent/100ml or CDC Any Quantity
Acetic	a	A
Propionic	p	P
Isobutyric	ib	iB
Butyric	b	B
Isovaleric	iv	iV
Valeric	v	V
Isocaproic	ic	iC
Caproic	c	C
Hepatanoic	h	H
Non Volatile Acids		
Lactic	l	L
Succinic	s	S
Alcohols	Not quantitated	
Ethanol	2	
Butanol	4	

Formic acid not included as only very large quantities were detected. Generally, detection of formic acid is difficult and as a result its production is not regarded as important in identification of anaerobes. Alcohols were not included in CDC characterisation key and were therefore omitted from Green Lane Hospital results using CDC key.

Table Metabolic Products from Peptone Yeast Glucose Detected by Gas Liquid Chromatography

MICRO-ORGANISM	*B. fragilis*		*B. vulgatus*		*B. asaccharolyticus*		*B. capillosus*		*C. bifermentans*		*C. difficile*		*C. histolyticum*	
Occurrence of Metabolic Products	Constant	Variable	Constant	Variable	Constant	Variable	Constant	Variable	Constant	Variable	Constant	Variable	Constant	Variable
VPI KEY	SAp	ibivl	SAp	ibivl	ABpibiv	sl	as	pl	A	picibivhbsl +24	ABibivic	pvcls	A	ls
G.L.H.* RESULT	SAp	iv	SAp		abpibiv	s	As	P	A	pic b +24	Abibivic	v +24	A	
VPI ⊙ TYPE SPECIES (From VPI Anaerobe Laboratory Manual)	SAP	ibivl	SAp	ibivl	Abpibiv	sl	AS	l	a	picibivhbsl +2	ABibivic	pvls +24	A	ls
CDC KEY	SAPL	iBiV	SAPL	iBiV	ABPiBiV		AS		AiC	iBPiVB	ABiBiViCV	P	A	
G.L.H.* RESULT	SAPL	iV	SAPL	iV	ABPiBiV		ASP		AiC	P B	ABiBiViCV	P	A	
CULTURE ORIGIN G.L.H.*	NCTC	8560	Wadsworth	1887	NCTC	9337	VPI 10874		NCTC 1340		NCTC 11223		CDC 1942	
VPI ⊙ TYPE SPECIES	ATCC NCTC NCTC	23745 8560 9344	ATCC	8482	ATCC ATCC NCTC	25260 27067 8945	Not given		ATCC 638		ATCC 9689		ATCC 6282 ATCC 25770	

profiles would be obtained. It is surprising that the VPI key has not been revised as the need for revision is clearly indicated by sample GLC profiles contained in the manual itself.

Both GLH and VPI type species of *C. difficile* produced alcohols not shown on the VPI key chart. Although there is not enough evidence to advocate exclusion of alcohols from the VPI identification key this might be advisable as the chromatographic methods used are relatively insensitive to the detection and separation of alcohols. It is noteworthy that the CDC key, which gave a better performance than the VPI key does not include alcohols.

The CDC key failed to give a correct characterisation on one occasion due to unexpected production of propionic acid. One explanation for this is that the medium from which the fatty acid metabolites are produced is not strictly defined and as a result variation in metabolic products can occur. For example if the constituent peptone is obtained from a meat digestion process (e.g. proteose peptone) it may contain varying amounts of lactic acid which stimulate some anaerobes to produce corresponding amounts of propionic acid (Holdeman *et al,* 1977)[1]. Until a chemically defined medium and specific conditions for culture and incubation are established GLC patterns obtained by either the VPI or CDC methods should not be regarded as absolute.

In this study when the VPI system was used the largest number of possible identities was 13 for *B. fragilis* and the smallest number was one for *C. bifermentans*. For the CDC system the largest number was 6 for *C. difficile* and the smallest one for *B. asaccharolyticus*. Hence, GLC profiles provide important clues to the identification of anaerobic micro-organisms. Used as an identification clue the GLC profile will decrease the number of tests needed for characterisation of a micro-organism by reducing the number of possible identities.

Identification of the bacteria in this study can be made without using GLC in the medical laboratory. Porschen *et al* (1976)[3], Stargel *et al* (1978)[4] and Talley *et al* (1979)[5]. The biochemical, serological and antibiotic susceptibility tests described by these authors will give a presumptive identification 24-48 hours after isolation. In comparison GLC analysis required a broth which was at least 48 hours old and most of the type cultures will also require biochemical and antibiotic tests before presumptive identification is possible. Therefore, GLC will not save time or give a characterisation sufficient for identification.

This study indicates that in the present state of knowledge the use of GLC for the presumptive identification of anaerobic bacteria in a clinical laboratory could be an unnecessary expenditure of equipment, reagents and time. If complete identification of anaerobic bacteria is considered necessary then GLC could reduce the number of tests required.

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National Immunohaematology Proficiency Survey (NIPS)

A Summary of Results

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On behalf of the Technical Sub-Committee of the Transfusion Advisory Committee

Introduction

This is the third summary of results to be presented for publication and covers the last four surveys—

NIPS 11 (November 1980)
NIPS 12 (February 1981)
NIPS 13 (May 1981)
NIPS 14 (August 1981)

As has been previously stated, it is not the intention of these summaries to pass judgement but rather to present the results and for individual laboratories and technologists to be aware of their shortcomings and take the necessary steps to correct their deficiencies.

A comprehensive summary and discussion of results is distributed to each participating laboratory after each survey which details results on a confidential basis and contains comments from the survey referees on the antibodies and/or abnormalities present.

NIPS 11

(a) *Grouping*

050 A_1 Rh Positive R_1R_1 Kell Negative
051 O Rh Positive $R_1{}^Wr$ Kell Negative
052 A_2 Rh Positive $R_1{}^ur$ Kell Negative
053 O Rh Positive R_1R_1 Kell Negative

Comments: Although the majority of laboratories correctly grouped and genotyped all four cells, some laboratories are still mistyping due to various reasons: misuse of reagents, interpretation of results, transposition of results and clerical errors being the commonest sources of error. It was noted in this survey that a total of 24 laboratories used commercially imported anti-e in the genotyping when this is obviously unjustified—none of the cells being E positive. If this is normal practice the cost and wastage is considerable.

The reporting of cells that are D^u's is still causing a problem. To reiterate—all donor cells that type as apparent "D" negative must be tested for D^u, and if subsequently found to be D^u positive must be reported as "Rh Positive"

i.e. D and D^u Negative—Rh Negative
D or D^u Positive—Rh Positive

(b) *Antibody Screening and Identification*

Serum 050—Contains anti-c

Comment: One laboratory failed to detect the presence of an atypical antibody.

Although some laboratories were unsuccessful in the antibody identification they all stated that they would normally refer such specimens to another laboratory, the practice adopted by other laboratories who were successful in the antibody identification.

(c) *Cross Matching*

Cell 051—Incompatible due to anti-c
Cell 052—Incompatible due to antic-c
Cell 053—Compatible

All laboratories, a total of 56, who completed the cross match section successfully detected the incompatibility between cells 051 and 052 and the serum 050, by the Indirect Coombs technique. However a total of 14 laboratories are still encountering problems with their enzyme techniques.

NIPS 12

(a) *Grouping*

054—A_1 Rh Positive R_1r, Kell Negative, Fy^a Negative
055—A_2 Rh Negative rr, Kell Negative, Fy^a Positive
056—A_1 Rh Positive R_2r, Kell Positive, Fy^a Negative
057—O Rh Positive R_1R_1, Kell Positive, Fy^a Negative

Comment: Although there were fewer errors in the grouping section of this survey compared to

earlier surveys, those errors that did occur were similar to those previously reported. The excessive unjustified usage of anti-e was again apparent.

(b) *Antibody Screening and Identification*

Serum 054—Contains anti-Fy^a and weak anti-Kell

Comment: One laboratory failed to detect the presence of an atypical antibody. All laboratories who attempted the antibody identification were successful in identifying the anti-Fy^a. However the anti-Kell was very weak, one referee failing to detect it even when tested with a homozygous KK cell. This weakness was unintentional by the survey organiser.

(c) *Cross Matching*

Cell 055—Incompatible due to anti-Fy^a
Cell 056—Incompatible due to anti-Kell (see above)
Cell 057—Compatible

Comment: Of the 57 laboratories who completed the cross match section, eleven were private laboratories who used the techniques employed for ante-natal screening. All 57 laboratories detected the incompability of serum 054 and cell 055. Thirteen laboratories detected the incompability due to the anti-Kell by Indirect Coombs technique and one found an incompatibility by enzyme technique. One laboratory found cell 057 incompatible by enzyme technique.

NIPS 13

(a) *Grouping*

058—O Rh Positive R_1r, Kell Negative, Kp^a Negative
059—O Rh Positive R_1R_2, Kell Positive, Kp^a Negative
060—O Rh Negative rr, Kell Negative, Kp^a Negative
061—O Rh Positive R_1r, Kell Negative, Kp^a Positive*

(* Kp^a type incorrectly reported on Summary)

Comment: Although the number of errors were relatively few, errors were still apparent. One laboratory reported cell 058 as D negative, although if confirmed for D^u status this should have been picked up. One laboratory having obtained a negative result with their anti-D and cell 061 reported it as $R_1{}^ur$. There is still an apparent wastage of valuable typing serum in that laboratories are continuing to type with anti-e when this is clearly not justified. Some laboratories are not performing reverse or serum groupings on the serum supplied and one laboratory does not use Anti-A+B.

(b) *Antibody and Screening Identification*

Serum 058—Contains anti-Kell and anti-Kp^a

Comment: All laboratories detected the presence of the atypical antibodies, however some failed to identify the anti-Kp^a because of the absence of a Kp^a positive cell on their panel. However those that failed to identify or misidentified the anti-Kp^a indicated that they would refer the specimen to another laboratory.

(c) *Cross Matching*

Cell 059—Incompatible due to anti-Kell
Cell 060—Compatible
Cell 061—Incompatible due to anti-Kp^a

Comment: All laboratories detected the incompatibilities between serum 058 and cells 059 and 061. One laboratory reported cell 060 as being incompatible but this may have been due to the presence of small fibrin clots in the serum.

NIPS 14

(a) *Grouping*

062—O Rh Negative rr, Fy^a Negative
063—O Rh Positive $R_2{}^ur$, Fy^a Positive
064—O Rh Positive R_2r, Fy^a Negative
065—O Rh Negative rr, Fy^a Positive

Comment: Errors were still made in genotyping, D and D^u typing of cells in this survey. The methods of reporting a cell that is D negative, D^u positive are still numerous.

(b) *Antibody Screening and Identification*

Serum 062—Contains anti-C, anti-D and anti-Fy^a

Comment: The majority of laboratories that attempted the antibody identification correctly identified the anti-D and the anti-Fy^a, however only 50% correctly identified the anti-C.

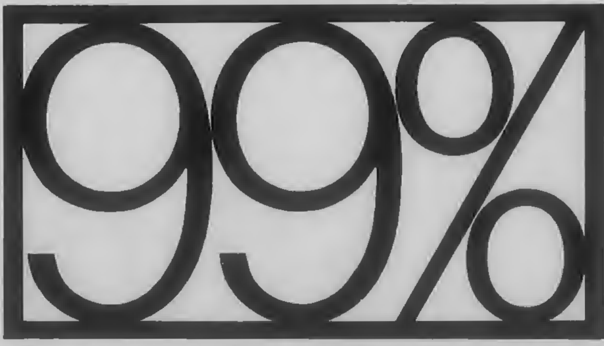
(c) *Cross Matching*

Cell 063—Incompatible due to anti-D
Cell 064—Incompatible due to anti-D
Cell 065—Incompatible due to anti-Fy^a

Comment: All laboratories detected the incompatibility between serum 062 and cell 064 by enzyme and Indirect Coombs technique. However 68% failed to detect the incompatibility between serum 062 and cell 063 by their enzyme techniques and some failed in their Indirect Coombs technique when using this weaker reactive cell. A number of laboratories also failed to detect the incompatibility between serum 062 and cell 065 due to the anti-Fy^a.

General Comments

The National Immunohaematology Proficiency Survey (NIPS) still remains a popular and



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

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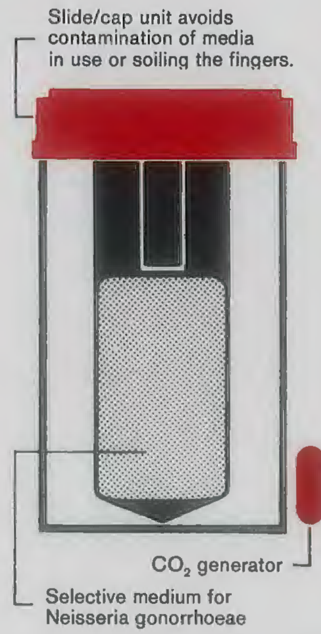

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informative external quality control system. Its straightforwardness is its greatest asset, the problems presented being within the scope of all laboratories. If more exotic antibodies were to be included the survey would lose some of its value as smaller laboratories would be unable to obtain a 100% correct answer and consequently perhaps they would lose interest.

Even in its simplicity a number of errors are still being made, not once has a 100% correct response been received.

The areas that still cause the organisers some concern are:

(a) the failure to carry our D^u testing on apparent D negative cell samples.

(b) the number of confusing ways a cell that is D negative, D^u positive is being reported.

(c) the problems some laboratories are encountering with their enzyme techniques.

(d) the wastage of valuable resources and reagents where the need is clearly not justified.

Those laboratories that feel they are not performing well are encouraged to contact their regional transfusion centre in order to seek out ways of improving their performance.

In conclusion the organisers would like once again to thank all participants for their continued support, their offers of raw material, and other helpful comments.

Acknowledgements

The organisers wish to thank Miss Gillian Kirk and Mrs Barbara England (Taranaki Base Hospital) for their patience in interpreting and typing the surveys distributed to date.

Technical Communication

Use of an Eppendorf Multipette 4780 with low Ionic Strength Saline suspended Red Cells

W. J. Henry ANZIMLT, Immunohaematology Department, Nelson Public Hospital

Received for publication June 1981

An important Immunohaematological technique involves using equal volumes of serum and L.I.S.S. suspended red cells. To overcome the occasional problems of electrostatically charged plastic test tubes and variations in drop volume using Pasteur pipettes we have introduced the new "Eppendorf Multipette" 4780 using the 0.5ml 'Combitip'.

The setting of "5" on the selector dial gives 8 increments of 50μl plus one priming delivery.

The 'Combitip' can be left on and rinsed out in water and saline in the usual manner as with a Pasteur pipette. Tips can be disposed of and replaced when worn or dirty.

It is more cumbersome than a Pasteur pipette and rather awkward to use until one gets accustomed to flicking the loading peg up and down with the thumb.

It is marginally slower than conventional methods. However I feel that any disadvantages in handling is outweighed by the confidence felt in knowing that the volumes are measured and that serum and cells are delivered into the bottom of the tube.

Preservation of 24 Hour Urine Collections

Alison Buchanan
Clinical Chemistry Department
Auckland Hospital

Sir,—We, and I believe many others, have been blindly following the instructions in standard textbooks [5, 7] for the preservation of 24-hour urine collections for the estimation of 5-Hydroxyindoleacetic acid. We were somewhat embarrassed to read [1, 3, 4] that glacial acetic acid interferes with the quantitative method of Udenfriend, [6] and with Goldenberg's[2] modification.

The extraction procedure involves ether extraction of the urine followed by a back extraction into a neutral buffer. Acetic acid, which is extractable into ether acidifies the buffer with consequent poor extraction of 5-Hydroxyindoleacetic acid. The percentage recovery decreases with increasing concentration of acid — approximately 40% being achieved with 25ml of glacial acetic acid in 2 litres of urine. We, as France *et al* (1981)[1] were not able to overcome the problem by increasing the strength of the buffer, and bringing the pH to 6.5 with sodium hydroxide before analysis gave even lower recoveries.

We have chosen 20ml of 6M hydrochloric acid as a preservative as this does not interfere with the extraction and is used routinely in this department for other 24-hour urine collections. Boric acid, 1g per litre is equally suitable.

REFERENCES

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2. Goldenberg, H., Specific Photometric Determination of 5-Hydroxyindoleacetic Acid in Urine. *Clin. Chem.* **19,** 38, (1973).
3. Johnson, R., Acetic Acid Interference with Determination of 5-Hydroxyindoleacetic Acid Obviated. *Clin. Chem.* **27,** 511, (1981) Letter.
4. Slaunwhite, D., Tuggey, R., Clements, J., Acetic Acid Interference with Determination of 5-Hydroxyindoleacetic Acid. *Clin. Chem.* **26,** 1754, (1980). Letter.
5. Tietz, N., *Fundamentals of Clinical Chemistry* 2nd ed., W. B. Saunders Company, Philadelphia 1976.
6. Udenfriend, S., Titus, E., Weissbach, H., The Identification of 5-Hydroxy-3-indoleacetic Acid in Normal Urine and a Method for its Assay. *J. Biol. Chem.* **216,** 499, (1955).
7. Varley, H., Gowenlock, A., Bell, M., *Practical Clinical Chemistry,* 5th ed., William Heinemann Medical Books Ltd., London, 1980.

Abstracts

Histology

Xylene Poisoning in Laboratory Workers: Case Reports and Discussion. Hipolito, Roberta. N. (1980), Laboratory Medicine. **11,** 9.

Little new about the dangers of inadequate ventillation in the histology laboratory; but five case studies which bring home the real dangers of overexposure to xylene.

A Multi-disciplinary Approach to Diagnostic Pathology. Wright, D. H. (1981), Med. Lab. Sci. **38,** 1.

A paper from the Albert Norman Memorial Lecture given at the 16th Triennial Conference of the Institute of Medical Laboratory Sciences, Bath, 8 September 1980. An often amusing paper which defines pathology as the study rather than diagnosis of disease. Dr Wright looks at lymphomas from a multi-disciplinary angle and hints at the importance of immunohistochemistry in the coming era of advancement in pathological knowledge.

The History of the Stain Commission (I); Preliminaries. Conn, H. J. (1980), Stain Technol. **55,** 269.

The first of a five part series recalling the history of the Biological Stain Commission. This part covers the events taking place until about 1920 and justly reads in part as autobiography.

High Affinity Binding of Horseradish Peroxidase to Collagenous Tissue in Formalin-Paraffin Processed Human Tissue. Kang Fan. (1980), Stain Technol. **55,** 307.

This paper reports on the results of a study to determine the reasons for the non-immunological binding of HRP and immunoglobulin bound HRP to collagen. Their reports demonstrate a very simple and highly selective method for the demonstration of collagen.

Hydroxyethylmethacrylate Embedding: An Improved Technique. Wynford-Thomas, D., Stringer, B. and Newman, G. R. (1981), Med. Lab. Sci. **38,** 121.

A method for achieving a higher polymerization success rate is described using anaerobic culture jars as polymerization chambers.

Combined Alizarin Red—Reticulum Stain for Tissue Localization of Calcium Deposits. Elbadawi, A.,

Musto, Linda. A. and Otto, M. L. (1981), Amer. J. Clin. Pathol. **75**, 355.

A combined alizarin red—reticulum stain for tissue localization of calcium deposits is described. The method allows precise localization of calcium deposits and crystals in relation to connective tissue elements.

A Word Processor Based Pathology Reporting System. Hieb, B. R. (1981), Amer. J. Clin. Pathol. **75**, 357.

This report describes a pathology report generating system based on a programmable word processing system.

The Robert C. Horn, Jr. Symposium on the Contributions of Surgical Pathology to Health Care. (1981). Amer. J. Clin. Pathol. **75** (Suppl), 443.

A collection of eleven papers. Titles include: an introduction to the history of surgical pathology; the future of research in surgical pathology; automated image analysis in clinical pathology.

A Hematoxylin and Eosin-Like Stain for Glycol Methacrylate Embedded Tissue Sections. Troyer, H. and Babich, Elaine. (1981), Stain Technol. **56**, 39.

A staining procedure is described for use with glycol methacrylate embedded tissue sections which does not stain the plastic embedment or remove the sections from the glass slides.

Effects of Fixation and Processing on Immunohistochemical Demonstration of Immunoglobulin in Paraffin Sections of Tonsil and Bone Marrow. Curran, R. C. and Gregory, J. (1980), *J. Clin. Pathol.* **33**, 1047.

A number of fixatives were tested to determine their suitability for use with the PAP method on tonsil and bone marrow sections. The necessity to trypsinise sections was examined as was the influence of a number of steps in the processing schedule.

A Non-Immunoglobulin Link Reagent for Use in the Unlabelled Antibody Method (PAP) of Immunohistochemistry. Watts, G. and Leathem, A. J. (1980), *Med. Lab. Sci.* **37**, 359.

A short paper describing the use of Protein A as an alternative to conventional antibody as a link reagent. The authors have found it to be more controllable in terms of standardisation of dilution thus overcoming the necessity to titrate the link reagent. A very economical reagent—1 mg may be diluted to stain 100,000 slides!

Obituary to Dr Ralph Lillie. Kasten, F. H. (1980), *Stain Technol.* **55**, 201.

Dr Lillie was regarded by many as the world's leading authority in histochemistry. This fine tribute reads as a history of histological technique since 1930 and is a comprehensive record of Dr Lillie's vast contribution. Many of his innovations seem nowadays to be simply commonsense.

Staining Myelin, Elastic Fibres and Nuclei with Iron Hematoxylin. Spooner, G. H., Reed, C. S. and Clark, G. (1980), *Stain Technol.* **55**, 185.

An attempt to learn something of the staining mechanisms involved with iron haematoxylin. Two new iron haematoxylin procedures were developed. Histochemical procedures demonstrated that amine groups are essential but the nature of the union between the tissue and iron haematoxylin complex was not determined.

Influence of Clearing Agents on Immunohistochemical Staining of Paraffin-embedded Tissue. Matthews, J. B. (1981), *J. Clin. Path.* **34**, 103.

A paper describing a study to determine the effect of different clearing agents on subsequent immunoreactivity of fixed and embedded tissue.

Effects of Different Fuchsin Analogs on the Feulgen Reaction. Teichman, J. S., Krick, T. P. and Nettleton, G. S. (1980), *J. Histochem. Cytochem.* **28**, 1062.

Basic Fuchsin from which Schiff's reagents used in the Feulgen reaction are usually prepared is a variable mixture of four triaminotriphenylmethane analogs. In this investigation Schiff's reagents prepared from pure fuchsin analogs were used to determine whether different analogs affect the absorbance of the Schiff's reagent-DNA complex formed in solution.

Electron Microscopy in Surgical Pathology: A Selective Review. Seymour, A. E. and Henderson, D. W. (1981), *Pathology* **13**, 111.

This review describes some of the characteristic morphology which can be demonstrated in neoplastic cells by electron microscopy and briefly considers several areas where the technique is of special value. A very fair appraisal of the role of the EM in the armoury of the surgical pathologist.

— B.C.T.

Microbiology

A Rapid Slide Coagglutination Test—An Alternative to the Fluorescent Antibody Test for the Identification of Neisseria Gonorrhoeae. Shanker, S., Daley, D. A. and Sorrell, T. C. (1981), *J. Clin. Pathol.* **34**, 420.

The authors tested the Phadebact Gonococcus Test (slide coagglutination test) for sensitivity and specificity and compared it with the fluorescent antibody test (Difco FA) which was the routine test in their

laboratory. The sensitivity of the Phadebact test was 96% compared to 85% using the FA test and the specificity was 94% compared to 88% for the FA test. 32 (10%) of the coagglutination tests could not be interpreted and the alternative method of testing, by heating a suspension of organism to 80°C for 20 minutes then retesting, was used. All but three of the isolates were then identified as *Neisseria gonorrhoeae.*

Four Hour Presumptive Identification of Enterobacteriaceae from Blood Cultures. Smith, E. G., Pritchard, Jane, K. and McCarthy, L. R. (1981), *Am. J. clin. Pathol.* **75,** 88.

The Minitek identification system was modified so that oxidase negative gram negative bacilli in blood cultures could be presumptively identified in four hours.

Rapid Flagella Stain. Forbes L. (1981), *J. clin. Microbiol.* **13,** 807.

A modification of the Leifson stain is shown to be reliable in staining flagella. Thoroughly clean slides are not necessary and the total staining time is four minutes. The stain can be kept at room temperature for up to two weeks.

Effect of Carbohydrate Content of Culture Media on Kovac's Oxidase Test, with Particular Reference to Vibrio spp. Jones, Anna M. (1981), *Med. Lab. Sci.* **38,** 133.

Pseudomonas aeruginosa, Vibrio parahaemolyticus and Vibrio species cultures were tested for their oxidase reaction on CLED, MacConkey, XLD, DCA, and TCBS agar. *Pseudomonas aeruginosa* and *Vibrio parahaemolyticus* gave positive oxidase reactions. However Vibrio cholerae species gave varying oxidase reactions because of the sucrose in the media, thereby requiring subculture to a media free from carbohydrates before the oxidase test could be done.

Comparison of Formalin-Ethyl Ether Sedimentation, Formalin-Ethyl Acetate Sedimentation and Zinc Sulfate Flotation Techniques for Detection of Intestinal Parasites. Truant, A. L., Elliott, S. H., Kelly, M. T. and Smith, J. H. (1981), *J. clin. Microbiol.* **13,** 882.

The formalin-ethyl acetate method was found to be equal to the formalin-ethyl ether sedimentation method in the detection of cysts, ova and larvae in faeces. It was also safer because it does not have the same fire hazard associated with the diethyl ether. Also demonstrated was that both sedimentation and flotation should be done as the zinc sulfate method detected protozoan cysts, *Hymenolepis nana* eggs and hookworm eggs better than the sedimentation methods.

Diagnosis of Bacteremia in Children by Quantitative Direct Plating and Radiometric Procedure. Scolea, L. A., Dryja, Diane, Sullivan, T. D., Mosovich, L., Ellerstein, N. and Neler, E. (1981) *J. clin. Microbiol.* **13,** 478.

Two thousand one hundred and twenty three cultures were tested by the BACTEC system, a Fisher Lederle bottle (broth with liquoid and PABA) and a quantitative direct plate method (QDP). The two main aims were to see the value of QDP and to evaluate the BACTEC for the early diagnosis of bacteremia, particularly with reference to *Haemophilus influenzae.* The main isolates were *Haemophilus influenzae, Neisseria meningitidis,* and *Streptococcus pneumoniae.* Direct plating of small quantities of blood (QDP) allowed the early detection of *H. influenzae* and *N. meningitidis* bacteremia in 88% of cases but in only 50% of cases of *S. pneumoniae* bacteremia were positive results obtained. *H. influenzae* grew well in the BACTEC broth but did not give a significant growth index on day 1 of incubation.

Evaluation of Transport Methods for Isolating *Shigella* spp. Wells, Joy, G. and Morris, G. K. (1981), *J. clin. Microbiol.* **13,** 789.

Buffered glycerol saline and Cary-Blair media held at room temperature, 4°C and frozen were compared with direct plating in the recovery of *Shigella* species from faeces. Direct plating was the method of choice but where there is a delay before culturing faeces, buffered glycerol saline was the better transport media. In addition refrigerated or frozen temperatures were better than room temperature for recovery of *Shigella* from both media.

Evaluation of Five Gentamicin Assay Procedures for Clinical Microbiology Laboratories. Selepak, Sally T., Witebsky, F. G., Robertson, E. A. and MacLowry, J. D. (1981), *J. clin. Microbiol.* **13,** 742.

The gentamicin assay techniques evaluated are a bioassay, an enzyme immunoassay (EMIT), a latex agglutination inhibition test, a fluorescence immunoassay (FIA) and a radioimmunoassay. Recovery and precision studies, time, cost and technical performance are all considered. For the authors laboratory the EMIT assay is chosen because it is precise, accurate, reasonably simple to perform and quick.

Detection of Yeast Septicemia by Biphasic and Radiometric Methods. Prevost, Elena and Bannister, E. (1981). *J. clin. Microbiol.* **13,** 655.

Over a three year period the Bactec 460 system and biaphasic brain heart infusion vented culture bottle were used to culture blood. The average detection times for the isolation of yeasts was 8.3 days for the biphasic method and 2.4 days for the radiometric method. The aerobic bottle of the Bactec 460 system was suitable for the isolation of yeasts, therefore there was no need for a special medium. However in some cases there was no positive reading on the Bactec 460 machine and blind subcultures appear necessary to recover some strains.

Differential Identification of *Mycobacterium fortuitum* and *Mycobacterium chelonei.* David H. L., Traore, I. and Fenillet, Antoinette. (1981), *J. Clin. Microbiol.* **13,** 6.

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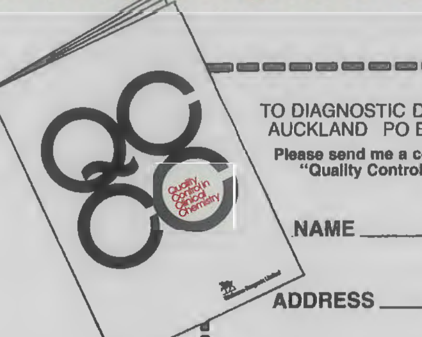

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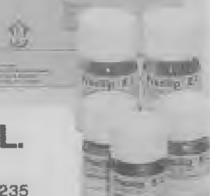
HDL-Chol., Women	< 65	45-65	< 45



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nitrate reductase, B glucosidase, acid production from fructose, penicillinase and trehalose the *Mycobacterium fortuitum* complex can be separated into two major groups *M. fortuitum and M. chelonei.*

Analysis of Tests and Differentiation *Tricophyton rubrum* **from** *Tricophyton mentagrophytes.* Sinski, J. T., Van Avermaete, D. and Kelly, L. M. (1981), *J. clin. Microbiol.* **13,** 62.

The *in vitro* hair perforation test was taken as the criterion for differentiating *T. mentagrophytes* and *T. rubrum.* As it is time consuming the authors compared nine other tests with it to find if any test or combination could differentiate the two species more quickly and just as reliably.

No test was found to be as reliable but using potato carrot agar for sporulation and only performing the hair penetration test on isolates that failed to produce spores was just as reliable and more expedient than in vitro hair penetration.

Iodometric Spot Test for Detection of Beta-Lactamase in *Haemophilus influenzae.* Lee, Wie-Shing and Komarmy, L. (1981), *J. clin. Microbiol.* **13, 224.**

This paper describes a simple test using penicillin G powder, filter paper and grams iodine to detect beta lactamase. As these are stable reagents and present in all laboratories it is useful where these tests are performed infrequently.

Gas Liquid Chromatography in Routine Processing of Blood Cultures for Detecting Anaerobic Bacteraemia. Reig, M., Molina, D., Loza, E., Lwdesma, Ma and Mesegner, Ma. (1981), *J. clin. Pathol.* **34,** 189.

Gas liquid chromatography was performed on 233 positive blood cultures to find which of the short chain fatty acids produced are the best anaerobe markers and whether these correlated well with culture results. GLC analysis and routine subculture were only done on blood cultures with macroscopic signs of growth. Butyric and isovaleric acids were the best anaerobe markers with a sensitivity of 88 percent and only one false positive (*Corynebacterium spp*).

The Diagnosis of Legionnaires' Disease by Counterimmunoelectrophoresis. Holliday, M. G. (1980), *J. clin. Pathol.* **33,** 1174.

Counterimmunoelectrophoresis showed excellent correlation with the indirect fluorescent antibody test (FTA) in detecting antibodies to *Legionella pneumophila* in sera from patients with Legionaires' disease. It was simpler than IFA and the antigen remained stable for up to six months at 4°C. Details of optimum conditions for the method are given.

The Development of the Bacterial Flora in Normal Neonates. Rotimi, V. O. and Duerden, B. J. (1981), *J. med. Microbiol.* **14,** 51.

Twenty-three babies were studied for the development of their bacterial flora in their first week of life.

Faeces and swabs from umbilicus and mouth were taken on the first, second, third and sixth days.

Staphylococcus aures was the predominant organism from the umbilicus, viridans streptococci predominated in the mouth and anaerobes predominated in faeces.

— S.G.

IMMUNOHAEMATOLOGY

Renal Transplant in a Patient with Major Donor Recipient Blood Group Incompatibility. Slapak, M., Naik, R. B., and Lee, H. A. (1980). *Transplantation* **31,** 1.

A 47-year-old patient with blood group O inadvertently received a mismatched kidney from a donor of blood group A. Two days after transplantation the clinical and biochemical manifestation of an intrarenal and intramuscular coagulation was seen. This was treated by plasma exchange with the rapid reversal of all parameters and reduction of both the IgG and IgM component of the circulating Anti A antibody.

The patient, 20 months after transplantation has normal renal function.

A High Incidence Antibody (Anti-Sc3) in the Serum of a Sc:-1, -2 Patient. Mason, S. G., Vengelen-Tyler, V., Cohen, N., Best, M. and Quirk, J. (1980). *Transfusion* **20,** 5.

An individual has been found whose red blood cells type as Sc:-1, -2, and do not absorb or yield on elution, Anti-Sc 1 or Anti-Sc 2. The antibody was called Anti-Sc 3 and was shown not to contain separable specificities when absorption studies were performed with Sc: -1, 2 and Sc: 1-2 red cells. It appears that all Sc: 1 or Sc: 2 red blood cells are also Sc: 3, while those that are Sc: -1, -2 are Sc -3.

Plasma Exchange for Haemolytic Crisis Due to Autoimmune Haemolytic Anaemia of the IgG Warm Type. Garelli, S., Mosconi, L., Vallonesi, M., Schieppati, G., and Naiassa, G. (1980). *Blut* 41, 387.

A patient with hyperacute haemolytic crisis due to AIHA of the IgG type was treated by combined plasmapheresis and exchange transfusion. During the post-exchange period, the auto-antibodies responsible for the haemolytic crisis, switched from IgG1 and IgG3 to IgG2 and IgG4.

Use of IgM-Hepatitis A Antibody Testing. Syndman, D. R. Diewstag, J. L., Stept, B., Brink, E. W., Ryan, D. M. and Fawaz, K. A. (1981), *JAMA* **245:** 8.

An outbreak of Hepatitis, type A, was investigated using traditional methods. In addition, serological tests for IgM and IgG antibody to Hepatitis A virus (HAV) were used. The solid-phase radio-immunoassay technique appears to be the most reliable, reproducible and adaptable to large scale application.

Donor-specific B and T Lymphocyte Antibodies and Kidney Graft Survival. Jeannet, M., Benzonana, G. and Arni, I. (1981). *Transplantation* **31:** 3.

The prognostic significance of a positive B and/or T cell cross-match test before transplantation was analysed in 174 cadaver kidney transplants. The results indicated, that at least two types of donor-specific B and/or T cell antibodies must be distinguished, those directed against HLA (A, B, C or DR) antigens and those against autologous B. lymphocytes, must be identified in order to relate the results to probable outcome of the graft.

Accidental Transplantation of Bronchial Carcinoma from Cadaver Donor to Two Recipients of Renal Allografts. Forbes, G. B., Goggin, M. J., Dische, F. E., Saeed, T. T., Parsons, V., Harding, M. J., Bewick, M. and Rudge, C. T. (1981), *J. clin. Pathol.* **34,** 109.

Malignancy was transferred to two patients, each of whom received a renal transplant from a cadaver donor who was found at necroscopy to have a small, clinically silent carcinoma of lung. Both recipients died with metastic bronchial carcinoma of the same histological type as the donor's tumour. An interesting review is presented.

New HLA-D Alleles Associated with DR1 and DR2. Suciu-Fola, N., Godfrey, M., Khan, R., Woodward, K., Rohowsky, C., Reed, E., Hardy, M. and Reemjsma, K. (1981), *Tissue Antigens* **17:** 294.

This study describes two new HLA-D specificities (1) LD13, associated with DR1; (2) LD14, associated with DR2.

Evaluation of Commercially Available Low Ionic Strength Salt (Liss) Solutions. Dynan, P. K. (1982), *Med. Lab. Sci.* **38,** 13.

A number of commercially available low ionic strength salt (Liss) solutions were tested using varied dilutions of selected antisera covering a wide range of antisera. Variation existed between results obtained emphasising that good quality control methods need to be employed.

Hepatitis Infection in Clinical Laboratory Staff. Grist, N. R. (1981), *Med. Lab. Sci.* **38,** 103.

A review is presented concerning the decreased reported incidences of Hepatitis B. A number of practical ways of avoiding accidental laboratory infection with Hepatitis B are discussed.

Delayed Haemolytic Episodes Due to Anti-M. Furlong, M. B. and Monaghan, W. P. (1981), *Transfusion* **21,** 1.45.

Three cases in which Anti-M, undetectable in pretransfusion serum, is reported as being responsible for accelerated haemolysis of cross-match—compatible red blood cells 5 to 15 days after transfusion. Haemolysis was mild and was not clinically suspected in any of these three patients until blood was requested for further transfusion. Anti-M was subsequently identified in both the serum and the eluate from the post-transfusion sample in two cases, on the third case, Anti-M was only detected in the post-transfusion sample.

A Successful Program of Immunizing Rh-Negative Male Volunteers for Anti-D Production Using Frozen/Thawed Blood. Urbancik, S. J. and Robertson, A. E. (1981), *Transfusion* **21,** 1.64.

Rh (D)-positive frozen/thawed red blood cells were used to immunise 28 Rh(D)-negative male volunteers. It is obvious from the results obtained that the use of frozen/thawed red blood cells for immunization has the advantage of permitting

optimum matching for undesirable red blood cell antigens and minimising the risk of transmitting disease of the recipients.

Delayed Haemolytic Transfusion Reaction Caused by Anti-P1 Antibody. Chandeysson, P. L., Flye, E. W., Simpkins, S. M. and Holland, P. V. (1981), *Transfusion* **21,** 1.77.

An interesting case is presented concerning a multi-transfused patient who underwent a delayed haemolytic transfusion reaction probably due to an anamnestic response to the P1 antigen.

The Histocompatibility (HLA) Antigen Distribution in Multiple Sclerosis Patients in Zimbabwe. Lowe, R. F., Moore, H. H., and Briggs, B. R. (1980). *Cen. Afr. Jnr. Med.* **26,** 11.

Forty Zimbabwean multiple sclerosis patients were HLA typed and their antigen frequencies compared to a larger control group. HLA-A29, B7 and B17 were shown to be increased in the patient group. HLA-A1 was decreased and HLA-A28 was completely absent.

Modification of Automated Coomb's Test System for Rapid Antigen Screening of Donor Blood. Munks, R. (1980). *Med. Lab. Sci.* **37,** 4.

By replacing the physiological saline normally used as the wash solution with low ionic strength saline (LISS) and replacing the anti-human-globulin (AHG) normally placed in the AHG cartridge in an automated Coomb's system, a system for rapidly grouping red cells can be set up.

Bg^a and Bg^b Correlations with HLA Antigens by Capillary Tube Technique. Crawford, M. N. and Schroeder, M. L. (1980). *Transfusion* **20,** 5.

The manual capillary tube technique, in a series of blood samples collected into CPD, demonstrated Bg^a on the red blood cells of 93% of the HLA-B7 donors and Bg^b on the red blood cells of 80% of the HLA-B17 donors. Successful results require normal red blood cell-plasma ratios in the samples and avoidance of Heparin for collection.

— L.M.M.

CLINICAL BIOCHEMISTRY

-Glutamyltransferase and Alkaline Phosphatase Activities Compared in Serum of Normal Children and Children with Liver Disease. Knight, J. A. and Hayward, R. E. (1981), *Clin. Chem.* **27,** 48.

Alkaline Phosphatase and Glutamyltransferase levels were measured in serum from children with various hepatic disorders, and also in the serum of normal children. While A.L.P. levels are raised in essentially the same disorders as the G.T.P. levels, the authors believe the G.T.P. assay to be a more useful and sensitive indicator of obstructive and infiltrative liver disease.

Rapid, Fully Automated Radioimmunoassay of Prostatic Acid Phosphatase in Serum. Dass, S., Bowen, N. L. and Bagshawe, K. D. (1980), *Clin. Chem.* **26,** 1583.

Described is a fully automated double-antibody radioimmunoassay for the measurement of Prostatic Acid Phosphatase in serum. Antibody was obtained after injecting rabbits with a purified P.A.P. obtained from the semen of normal males. The P.A.P. was radiolabelled with ^{125}I using the chloramine T method. Serum from patients with Prostatic Ca. and several control groups were measured and the lower detection limit was 2.0 ug of P.A.P. per litre of serum. Patients with Prostatic Ca. showed serum levels of up to 300 ug.

Combined Enzymatic-Jaffe Method for Determination of Creatinine in Serum. Masson, P., Ohlsson, P. and Bjorkhem, I. (1981), *Clin. Chem.* **27,** 18.

Described is a modification of a combined Enzymatic-Jaffe reaction which showed falsely low results when using a purified enzyme. This effect was caused by the reversal of the reaction due to Creatine. This is shown to be eliminated by conversion of Creatine to Creatine Phosphate. The modification is evaluated.

Acetylcholinesterase and Fetal Malformations: Modified Qualitative Technique for Diagnosis of Neural Tube Defects. Haddow, J. E., Morin, M. E. Holman, Mary, S. and Miller, W. A. (1981), *Clin. Chem.* **27,** 61.

Measurement of an isoenzyme of acetylcholinesterase in amniotic fluid samples by polyacrylamide gel electrophoresis can decrease the proportion of false positive results from the alpha-fetoprotein assay. Described is the methodology and the results obtained from a study of 214 amniotic fluid specimens from pregnancies of known outcome.

A Study of the Use of Polyethylene Glycol in Estimating Cholesterol in High Density Lipoproteins. Demacker, P. N. M., Hijmans, A. G.

M., Vos-Janssen, H. E., Van't Laar, A. and Jansen, A. P. (1980), *Clin. Chem.* **26,** 1775.

Precipitation of Lipoproteins other than the HDL fraction using polyethylene glycol-6000 is described. The polyethylene glycol-6000 did not interfere with two enzymatic assays for cholesterol, or with the Huang *et al.* and Abell-Kendell methods. Results compared with those by Ultracentriguation and would appear to be the method of choice when testing lipaemic sera or with use with enzymatic cholesterol methods.

Enzyme Immunoassay of Estriol in Pregnancy Urine. Korhonen, M. K., Juntunen, K. O. and Stenman, U. (1980), *Clin. Chem.* **26,** 1829.

The method measures total urinary estriol with results showing good correlation with the Radio-immunoassay method. The E.I.A. method described would appear to be more efficient than the R.I.A. method when more than 20 samples are to be assayed.

Serum Iron and Total Iron-Binding Capacity Compared with Serum Ferritin in Assessment of Iron Deficiency. Frank, P. and Wang, S. (1981), *Clin. Chem.* **27,** 276.

Serum Iron, Total Iron-Binding Capacity (T.I.B.C.) and Transferrin Saturation values on 250 specimens are compared with Ferritin values. Results show that the Ferritin assay is useful for detecting Iron Deficiency when serum Iron and TIBC are not positively indicative. It is recommended that the Ferritin test be used as a follow-up test in these cases.

N.L.

HAEMATOLOGY

Graphing the Frequency Distribution. McGregor, A. J. (1980), *Journal of Audiovisual Media in Medicine* **3,** 17.

The author discusses the design of frequency distribution graphs and suggests that close attention to several small points—zero baseline, equal intervals, and number and size of intervals—can distinguish a meaningful graph from a misleading or confusing one.

Unsuitability of Evacuated Tubes for Monitoring Heparin Therapy by Activated Partial Thromboplastin Time. Heyns, A. duP., vanden Berg, D. J., Kleynhans, P. H. T., and du Toit, P. W. (1981), *J. Clin. Pathol.* **34,** 63.

The authors results show clearly that the monitoring of heparin therapy with the APTT is unreliable and hazardous if blood samples are collected in Vacutainer or Venoject tubes. They suggest that samples should be taken with a polystyrene syringe and transferred to a citrated polystyrene tube.

Significance of Phi Bodies in Acute Leukaemia. De Salvo Cardullo, L., Morilla, R. and Catovsky, D. (1981), *J. Clin. Pathol.* **34,** 153.

Phi bodies were demonstrated in acute myeloid leukaemia blasts only when 3, 3 diaminobenzidine was used as a substrate for the peroxidase stain. Like Auer rods Phi bodies appear to be characteristic of immature myeloid cells in leukaemia but are seen with a higher frequency than Auer rods in acute myeloid leukaemia.

Incidence of Infections in Patients with Pelger-Huet Leucocyte Anomaly. Vaya, A. and Aznar, J. (1980), *Br. J. Haemat.* **46,** 623.

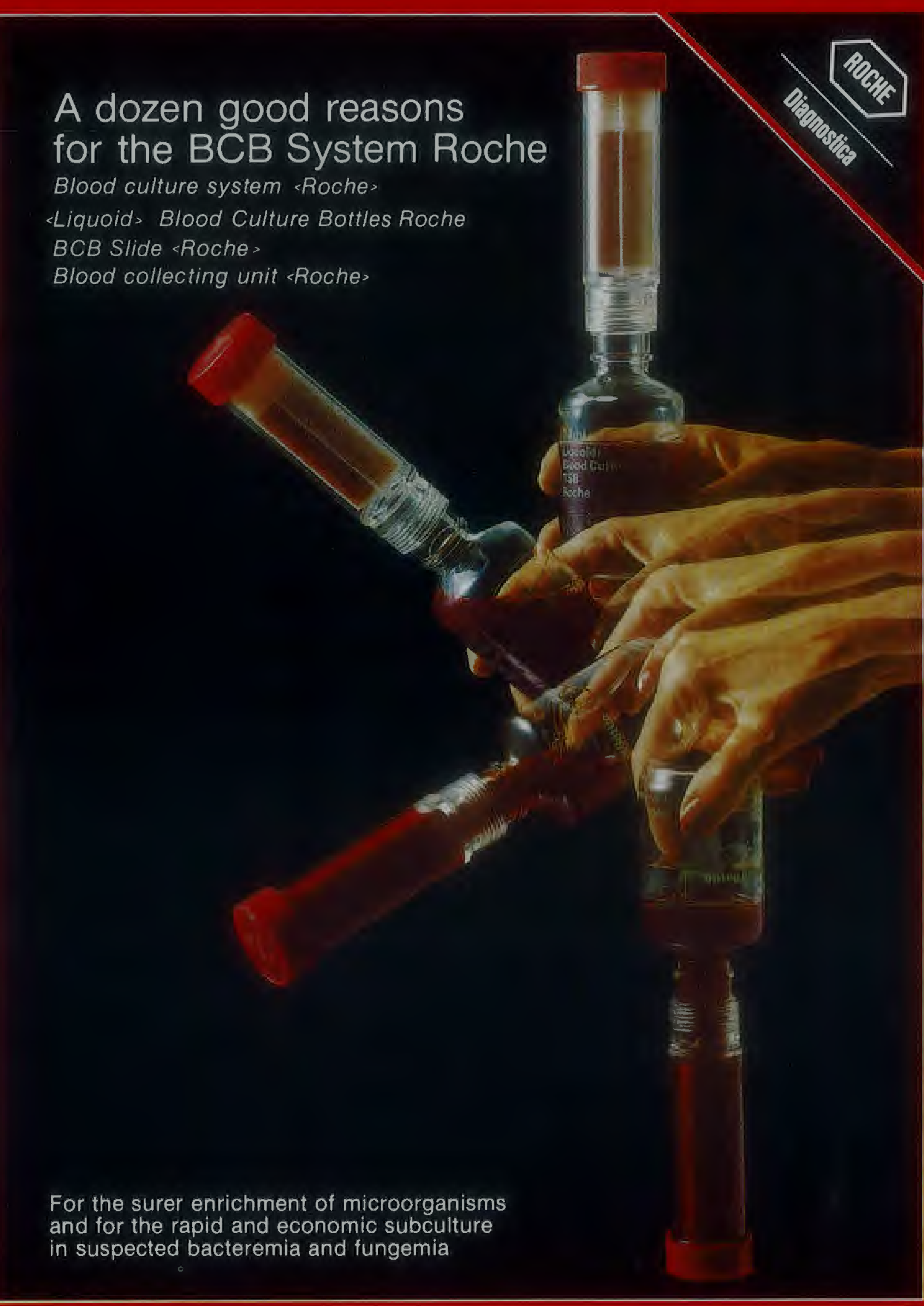
The authors have observed 85 subjects with the Pelger-Heut anomaly and found that the incidence of infections, particularly of a local nature, appeared to be higher than a control group. They suggest that a neutrophil migratory defect could be the basic cause of the increased incidence of local infections in Pelger-Huet patients.

Haematology Reference Values. Analysis by Different Statistical Techniques and Variations with Age and Sex. Giorno, R., Clifford, J. H., Beverly, Shirley and Rossing, R. G. (1980), *Am. J. Clin. Pathol.* **74,** 765.

The authors have collected the following haematologic parameters in 1744 healthy men and women aged 16 to 89 years: white cell count, red cell count, haemoglobin, haematocrit, mean cell volume, mean cell haemoglobin and mean cell haemoglobin concentration. They found statistically significant differences in reference values when using parametric and non-parametric values, significant sex differences for all values and significant changes with age to some of the values.

Autoimmune Diseases. Rose, N. R. (1981), *Scientific American* **244,** 70.

The author reviews autoimmunity, including the recognition of autoimmunity, components of immune response and functions of T and B lymphocytes including T cells as regulators.



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Activated Partial Thromboplastin Time. A Multicentre Evaluation of 11 Reagents in the Screening of Mild Haemophilia A. Italian Study Group (1980), *Scand. J. Haematol.* **25,** 308.

An internationally standardised preparation and 10 commercial kits widely used to perform the activated partial thromboplastin time were compared in four laboratories for the purpose of assessing their ability to detect mild deficiencies of factor VIII activity. An analysis of variance of clotting times showed significant differences between reagents and laboratories. Most of these kits are available in New Zealand.

The Euglobulin Clot Lysis Time, a Rapid and Sensitive Method for the Assay of Fibrinolytic Activity after Venous Stasis. Nordby, E., Arnesen, H., Andersen, P. and Godal, H. C. (1980), *Scand. J. Haematol.* **25,** 407.

For the estimation of fibrinolytic activity in euglobulin precipitates after venous stasis, the euglobulin clot lysis time proved to be as reproducible and probably even more sensitive than the fibrin plate method, and is therefore quite suitable for routine screening of fibrinolytic activity after venous stasis.

Classification of Acute Leukaemia by Light and Electron Microscope Cytochemistry. Jansson, S. E., Gripenberg, J., Vuopio, P., Teerenhoui, L. and Andersson, L. C. (1980), *Scand. J. Haematol.* **25,** 412.

Blast cells in 33 cases of adult acute leukaemia were investigated for signs of myeloid differentiation by light (Sudan Black B) and electron (peroxidase activity) microscope cytochemistry. All cases which were strongly sudan positive were also peroxidase positive and electron microscopy is of no further value in these cases. In 11 cases the blast cells were sudan negative and in eight cases they were weakly sudan positive: the results of the electron microscopical analysis would have altered the classification in nine of these 19 cases. The authors conclude that ultrastructural demonstration of peroxidase activity in blast cells is of value in the classification of acute leukaemia in cases of blasts either negative or only weakly positive for sudan.

Acute Appendicitis and the Leucocyte Count. Singh, K. J. (1980), *Med. J. Malaysia* **34,** 314.

The total and differential leucocyte counts were estimated in 100 consecutive cases operated for acute appendicitis. Leucocytosis was seen in 52 percent of cases and 48 percent of patients had a leucocyte count below 10 × 10-9/L. These results show a far lower percentage of Leucocytosis than many other published series.

Monitoring of Oral Anticoagulation by an Amidolytic Factor X Assay. Lammle, B., Bounameaux, H., Marbet, G. A., Eichlisberger, R. and Duckert, F. (1980), *Thrombos. Haemostas (Stuttgart)* **44,** 150.

The authors confirm previous reports of good correlation between the prothrombin time and the amidolytically (chromogenically) determined factor X assay. They suggest that a multicentre study now be carried out, especially to see if interlaboratory differences can be significantly removed with this factor X assay.

— E.R.C.

Book Reviews

Text Book for Laboratory Assistants. 3rd Edition I. A. Oppenheim (1981); Publisher C. V. Mosby, 187 pages, illustrated. $22.25. Available from Peryer Educational Books (S.I.) Ltd, P.O. Box 833, Christchurch.

This revised, soft covered text consists of eight chapters covering the major laboratory disciplines.

All aspects of clinical micobiology are mentioned to give a brief understanding. Because of the large number of topics covered the information on each is brief but useful. The section on urine bacteriology is disappointing. Examination of centrifuged urined is well covered but only scant reference is given to quantative bacteriology of urine. Few detailed methods or principles for specific tests are mentioned and important information concerning the formulae for media and its specific uses are often omitted.

This book has been written for the American Laboratory Assistant and as such the regulations governing the selection and bleeding of donors and the labelling and storage of donations differ from those used in New Zealand and therefore any potential purchaser should be aware of this limitation.

The section dealing with blood banking has

been reasonably well written and as such may be of some use as a basic introduction to the subject. Potential qualified technical assistants would still need to supplement their reading with standard reference books in order to understand the subject to a greater depth as the syllabus requires.

In general this book spends too much time in unimportant subjects and there is a lack of basic information concerning safety, the use of automated equipment and the use of general laboratory equipment.

The basic and updated material would be useful to the novice Q.T.A.

Jenny Bristol, Bruce Lockwood, Alan Knight and Les Milligan.

Colour Atlas and Textbook of Diagnostic Microbiology.
Koneman, Allen, Dowell and Sommers.
Publisher: J. B. Lippincott. Four hundred and ninety-five pages. Price $US39.75.

This book was first published in 1979.

The authors have the following backgrounds: Stephen Allen and Herbert Sommers are clinical microbiologists. Elmer Koneman is the Executive Director, Colorado Association for continuing Medical Laboratory Education and V. Dowell is the Chief of the Enterobacteriology Branch, Centre for Disease Control, Atlanta.

The book has been written to introduce medical technologists and others interested in microbiology, to the practical laboratory identification of the more commonly encountered micro-organisms, associated with infectious disease. It is not intended to supplant current text books, but rather to show the student some of the different approaches which may be used to provide relevant results.

The book contains many tables, and numerous charts interspersed through the text. The charts give a concise step by step account of the theory, procedure and interpretation relating to the particular topic. Much of the subject matter is illustrated with colour prints, black and white photographs and line drawings. In all there are 39 colour plates each containing between six and twelve colour prints. The majority of the prints are excellent.

The book is divided into the following chapters, Introduction to medical microbiology, the *Enterobacteriaceae,* the non-fermentative Gram negative bacilli, the unusual Gram negative bacilli, *Haemophilus,* packaged medical identification systems, the Gram positive cocci, *Neisseria,* the Aerobic gram positive bacilli, the anaerobic bacteria, anti-microbial susceptibility testing, mycology, and parasitology.

Some topics are dealt with in considerable depth while others receive fairly superficial treatment. Forty pages for example are devoted to the *Enterobacteriaceae,* while the fourteen genuses, including *Brucella* and *Vibrio,* listed under the unusual Gram negative bacilli, are covered in just sixteen pages.

The chapters devoted to the non-fermentative Gram negative bacilli, packaged microbial identification sytemsa and anaerobic bacteria should have a wide appeal.

There is little to criticise in this book. An important exception would be the technique used to inoculate media used for biochemical tests. Direct inoculation from selective culture media without sub-culture first on to a non-selective plate, or at least the confirmation of a pure growth with a purity plate was surprising. Occasionally I would have expected principles to be explained in greater depth.

Overall I found much to commend this book. I liked the format which is ideal for student use. It has sufficient depth to be of value to the more experienced microbiologist. It is an attractively produced volume.

I would recommend this book to all technologists with an interest in medical microbiology.

A.F.H.

Biochemical and Biological Applications of Isotachophoresis. Edited by Albert Adam and Carlo Schots. Analytical Chemistry Symposia Series—Volume 5. Elsevier Scientific Publishing Co., Amsterdam, Oxford, New York, 1980. Two hundred and seventy-eight pages, hard cover.

Isotachophoresis (ITP) is a high-resolution electrophoretic technique by which charged molecules are separated on the basis of differences in net-mobility. During ITP sample constituents are concentrated and migrate in sharply defined zones between a leading ion of high mobility and a terminating electrolyte of low mobility. The technique is thus analagous to the stacking gel of the more familiar disc-gel electrophoresis system of Ornstein and Davis. Biochemical and Biological Applications of Isotachoporesis is the title of the edited proceedings of the First International Symposium held in Baconfoy, Belgium, 4-5 May 1979, and as such is set out in typical rapid journal style with papers appearing as printed reproductions of the original typescript. The book begins with an introductory paper that includes a thorough mathematical discussion of the theoretical aspects of the topic. The rest of the text is essentially devoted to the applications of IPT. Papers of chemical pathology interest include: the determination of urinary oxalate, ITP of uremic metabolites including the determination of serum uric acid, the determination of low-molecular weight carboxylic acids (lactic, pyruvic, hydroxybutyric and acetoacetic) in biological samples and ITP of cerebospinal fluid proteins in neurological disease.

Since ITP has yet to find widespread use in clinical chemistry laboratories this book would be of little value in laboratory libraries as a teaching aid or "methods" manual. It might, however, be ready by experienced medical laboratory scientists wishing to be informed of recent developments and applications of ITP.

P. L. Hurst

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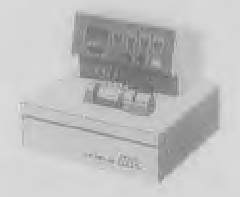

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GUIDE TO MEDICAL LABORATORY INSTRUMENTS by C. D. FERRIS (1980) CLOTH BOUND 260 pages. Little Brown & Co $25.95. Available from Australia and New Zealand Book Co. Pty, 2/10 Colway Place, Glenfield, Auckland 10.

This book is written for junior year students in the Medical Technology Program at the University of Wyoming. It is relatively short at 260 pages and could have been a great deal shorter without loss of substance, if the Introduction and Summaries had been left out. For example in the chapter on Electrophoresis, the second paragraph is largely a repetition of the first paragraph, and having read the chapter on Ion Selective Electrodes, the summary tells one that one has just read the chapter on Ion Selective Electrodes.

In spite of this there is much useful information on a great variety of laboratory instruments including Particle Counters and Nuclear Counters and a good chapter on Basic Electricity. For New Zealand readers the book falls between two stools. The treatment is too mathematical and detailed for most trainees in our present system and I think it leaves too many questions unanswered to be a University Text. Because of this I think it would he a useful addition to the laboratory shelves rather than to a trainee's library.

W.D.O.

Books Received

MYCOBACTERIA (Institute of Medical Laboratory Sciences Monographs Series) Maureen V. Chadwick. John Wright and Sons, 42-44 Triangle West, Bristol BS8 1EX England. 186mm x 123mm, 114 pages. U.K. Price £ 5.00 publishing date 24 September 1981.

Cumulative Index to Nursing and Allied Health Literature including a list of Subject Headings 1980 Vol. 25. De Launa LOCKWOOD Editor Glendale Adventist Medical Centre, P.O. Box 871 Glendale California CA 91209 United States of America.

Errata

Vol. 35 No. 2 Page 65.
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