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ISSN 0028-8349

THE NEW ZEALAND JOURNAL OF

medical laboratory technology

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

VOLUME 35, No. 1

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The New Zealand Journal of Medical Laboratory Technology

Volume 35, No. 1 March, 1981

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

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

Intending contributors should submit their material to the Editor, C/o Immunohaematology Department, P.O. Box 946, Public Hospital, Dunedin, New Zealand. Acceptance is at the discretion of the Editor, and no undertaking is given that any article will be published in a particular issue. The copy deadline for each issue is the first of the month prior to the month of publication.

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A Pericentric Inversion of Chromosome 12 in Metamorphosis of Chronic Granulocytic Leukaemia

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Received for publication December 1980

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3. Cytogenetic Unit, Palmerston North Hospital, Palmerston North, New Zealand.

Summary

The unique finding of a pericentric inversion of chromosome number 12 is described in relationship with metamorphosis of C.G.L. The other chromosome anomalies present are trisomy 8, a dicentric isochromosome for the long arm of 17, and the Philadelphia 9q:22q translocation.

Introduction

A well known pattern of chromosome evolution associated with chronic granulocytic leukaemia has become evident over the past decade. As well as chromosome 9 being a receptor for the translocated segment of 22, (Ph[1] chromosome) numbers 2,3,4,6,10,11,13,16,17,21 and 22 have all been involved, albeit rarely.

In metamorphosis of the disease the most frequent chromosome patterns found in association with the 9q:22q translocation, either together or separately, are trisomy 8, trisomy 19, the substitution of an isochromosome 17q for one of the number 17 chromosomes and an additional Ph[1] chromosome. Fairly common, too, are the findings of extra balanced reciprocal translocations, as well as the Ph[1] chromosome and its various receptors. This paper reports the unique finding of a pericentric inversion of a number 12 chromosome in connection with metamorphosis of C.G.L. This would appear to be the first report of an inverted 12 to be found in any disease where chromosomal abnormalities can be shown to be present.

Case Report W.A.

Aged 25 years, presented in May 1973 with tiredness. He was found to have a very large spleen and a white cell count of 100×10^9 per litre with the following differential: neutrophils 35%, lymphocytes 5%, eosinophils 12%, basophils 10%, metamyelocytes 10%, myelocytes 18%, promyelocytes 6%, myeloblasts 4%. Haemoglobin was 140 g per litre and platelets 500×10^9 per litre. A leucocyte alkaline phosphatase score of 2, hyperplastic granulopoiesis in the marrow plus the presence of a Philadelphia chromosome confirmed the diagnosis of chronic granulocytic leukaemia. Busulphan corrected the splenomegaly and blood picture, and he remained well until March 1978 when he developed marrow hypoplasia. The busulphan was stopped. He required periodic transfusions over this period, and the blood picture returned to normal by September 1978. In November 1978, the rapid development of a very large spleen heralded the appearance of a blast cell crisis. At this time the white cell count was 18.2×10^9 per litre (11% neutrophils, 14% lymphocytes, 1% eosinophils, 46% basophils, 1% myelocytes, 2% promyelocytes, 25% myeloblasts). Haemoglobin was 66 g per litre and platelets 16×10^9 per litre. His marrow was hypercellular, containing mainly blast cells with PAS-positive granules in many of these cells.

W.A. died in May 1979.

Chromosome Studies

Chromosome investigations were done when the patient was haematologically diagnosed as being in the blast phase of C.G.L. Cultures from both non-PHA- and PHA-stimulated peripheral blood were examined.

In the non-PHA cultures, two cell lines were present. Twelve percent of the cells screened showed a 46XY t(9;22)(q34:q11), inv(12)(p13;-q13). The other 88% screened showed a 47XY, +8, t(9;22)(q34;q11), inv (12)(p13;q13), i dic (17q)(p11). One hundred cells were screened. Figure 1).

The PHA-stimulated cultures gave the same chromosome findings as the non-PHA, with no normal cell line in evidence. Repeat cultures were performed just prior to death in May 1979 but, again, no normal cell line was seen.

C banding confirmed the isochromosome 17q to be dicentric (Fig. 1).

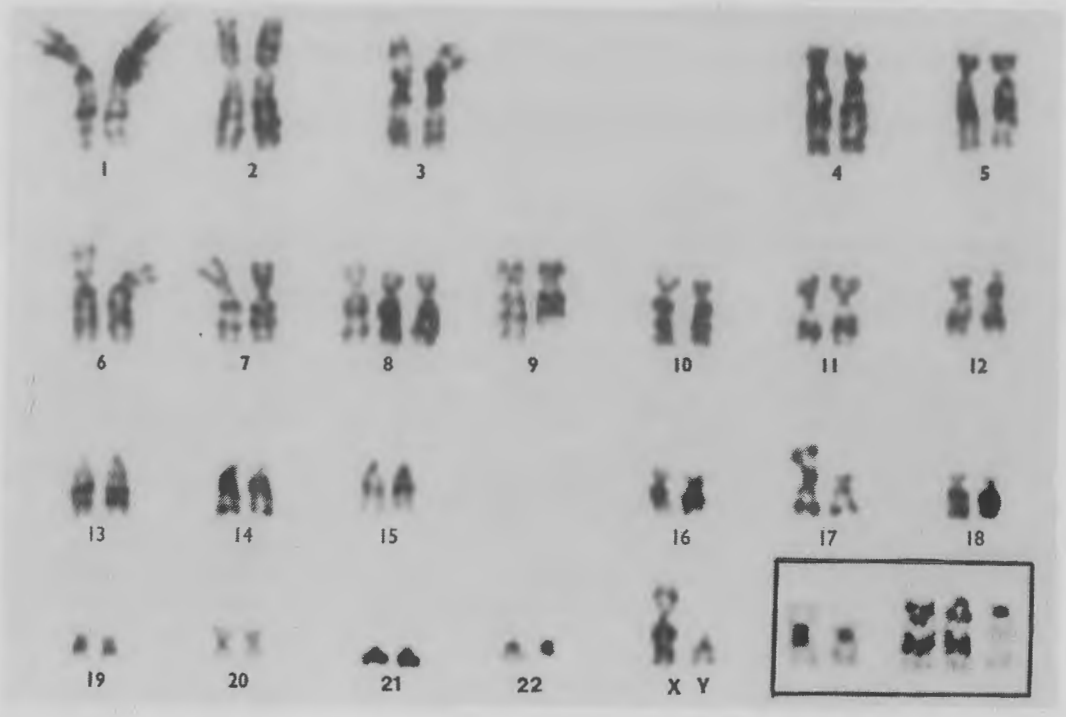

Figure 1.—G banded karyotype of Patient 47XY, +8, t(9q:22q) inv (12), i dic (17q).

Further example of inv (12) and C banded preparations of iso 17q and inv (12).

Discussion

The Philadelphia 9q;22q translocation appears to have the typical accepted break points q34;q11, and the dicentric isochromosome 17q would seem to have the break point for its formation located in the short arm 17p11.

The position of this break point in relationship to the dicentric 17q isochromosome has already been speculated on by McDermott *et al.* (1978)[1], but it would be informative to see other reports appearing in the literature with regard to the specificity of this break point location, especially in the light of recent publications. Page and Watt (1978)[2], Rowley and de la Chapelle (1978)[3].

The pericentric inversion of 12 was demonstrated in both clone lines isolated, one of which did not possess trisomy 8 or the isochromosome 17q, indicating the inverted 12's appearance before the other commonly found anomalies seen.

It was not possible to demonstrate the inversion as a carrier state as no normal cell line was found. The patient was adopted at the age of six months, no parental studies could be performed, and skin biopsy was not possible. The patient's two children were investigated, however, and found to be negative for the inverted 12. There was no history of miscarriages in the family.

It can be seen by study of the literature that, while some pericentric inversions confer a significant risk, others are so benign and occur so frequently that they are now considered normal chromosomal variants within the population. Trunca and Opitz (1944)[4].

In our particular case, as the frequency of structural aberrations is a consistent feature in metamorphosis of C.G.L., the origin of the inverted 12 was more than likely the result of the patient's clinical condition and not present in a normal carrier state although, as has been stated, this cannot be excluded with any certainty.

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The Use of Donor Blood as a Haematology Control for the Coulter S

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Green Lane Hospital Laboratory

Received for publication September 1980

Summary

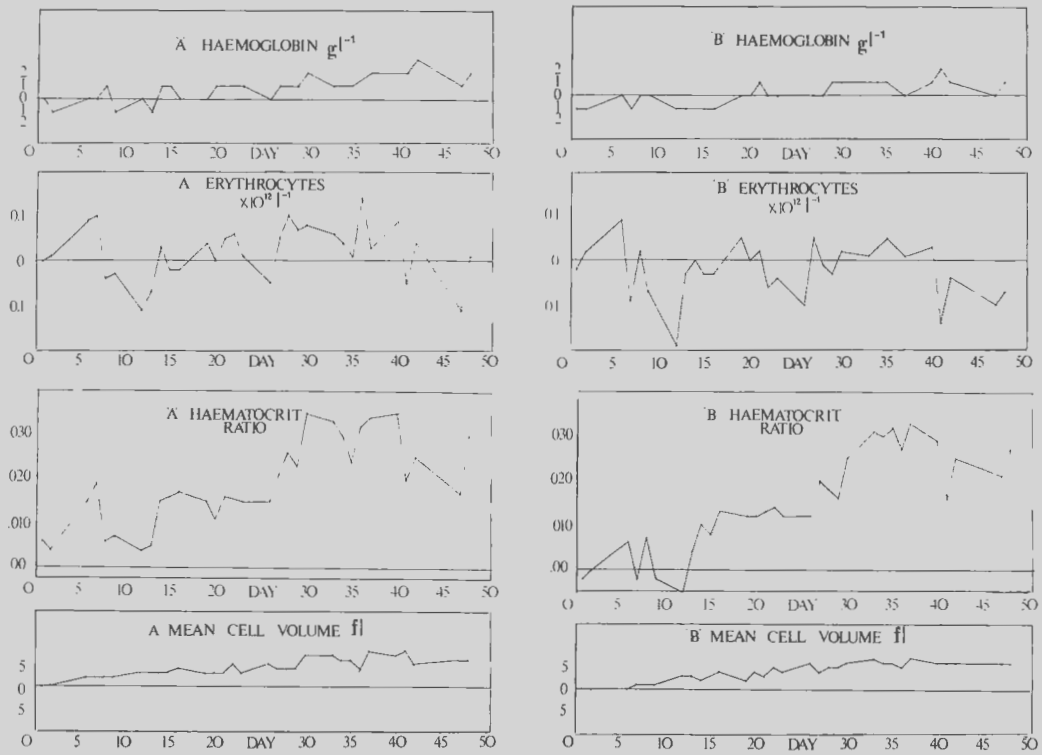
This paper evaluates the use of untreated "donor blood" as a control for routine use. It shows that donor blood undergoes changes in some parameters and remains stable in others for periods of at least three months. It also shows that the changes are very linear, beginning after collection and not changing appreciably over a three month period.

Introduction

Quality control of automated blood counting systems is an essential part of the activity of a haematology laboratory. Effective quality control requires the inclusion of control samples in the routine test procedure. Britten *et al.* (1969)[1], Overton (1971)[2], Cavill and Jacobs (1973)[3]. Application of this is limited by lack of suitable control preparations. Existing commercial preparations are expensive and therefore most laboratories prepare their own control samples for routine use. Some laboratories use time-expired donor blood as a control, therefore the object of this exercise is to determine whether fresh or time-expired donor blood is suitable for a control and for how long would this control be stable.

Materials and Methods

Two group A, CPD units of blood were obtained from the Blood Transfusion Service (Auckland).



GRAPH "A"
Donor Blood 3-50 days

GRAPH "B"
Donor Blood 31-78 days

The values on the graphs are expressed as relative deviations from the means.

Unit A (Graph's "A") was fresh donor blood, three days old.

Unit B (Graph's "B") was time-expired donor blood, 31 days old.

Each unit of blood was dispensed as aseptically as possible into covered autoclaved flasks, and then homogenised gently for one hour on a magnetic stirrer. No bacteriostatic agents were added. The blood was then dispensed aseptically through a transfusion "giving set" (a filter is necessary if not using a giving set) into 5 ml plain Vacutainer tubes. These tubes were stored vertically at 4-10°C and not disturbed until required. During the storage the red cells sediment and this makes resuspension difficult. Continuous gentle mixing for 30-60 minutes is needed to resuspend the material evenly. Subsequent resuspension for the following day only required about five minutes mixing, but before sampling it is important that the control has returned to room temperature.

Results and Discussion

Mean determinations: These were performed before refrigeration by taking tubes from the beginning, middle and end, and performing triplicate determinations, which were averaged to give the means. Sampling: All sampling was performed on the Green Lane Hospital routine Coulter S5. Each control tube was sampled twice and the second result only was plotted.

Graphic Interpretations

Graph "A" beginning as fresh blood 3-50 days.

Graph "B" beginning as time-expired blood 31-78 days.

It is important to note that there is an overlap. This evaluates the changes in bloods stored under control conditions for two months, and shows that no different changes occurred if the blood was one month older (unit B). This suggests that the blood could be stored under control conditions for at least two months.

(i) Leucocyte count: The count values are so low that carryover becomes significant when treated in a patient batch. Also, the values are not high enough to be considered of control worth.

(ii) Erythrocyte count: This control in both cases was considered stable when compared with the variations in a commercial stabilised control.

(iii) Haematocrit and Mean Cell Volume: Show gradual increases in both cases (slopes of "A" and "B" are the same) showing that the slope of deterioration is dependent on time and not the age of the unit, over the two month period, i.e. comparison of the two graphs which are of different ages.

(iv) Haemoglobin: Stable.

It is obvious from the graphs that the erythrocytes undergo a gradual deterioration seen by an increase in MCV. The increase in volume may be due to deterioriation of the cell membrane, with the cells swelling when placed in the diluent rather than the cells gradually swelling during storage.

If this type of control is used by a laboratory then it could be used for a period of up to two months or even more (if sufficient sample). Over this time the parameters show a standard linear deterioration regardless of age. If this type of control is to be used then it would be necessary to redetermine the mean values on a weekly or fortnightly basis.

There is no advantage in using fresh donor blood nor is there an advantage in continual preparations because the parameters do not appear to stabilise over a period of time, but show a regular deterioriation from the date of collection.

The leucocytes of donor blood for control purposes are unsatisfactory. Therefore, they can either be eliminated as a parameter or a stabilised preparation of cells may be added to serve as the leucocyte count.

Summary

I believe that the unstabilised units are not suitable as controls and the individual laboratory should try to find a better alternative. One such alternative is suggested by Morgan L. O. *et al.* (1978)[4]. This article uses time expired donor blood stabilised with glutaraldehyde which is suitable for the Coulter Model S.

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Hb D (Punjab)/β Thalassaemia Minor — A Case Report

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Received for publication, September 1980

Summary

The presence of a mild hypochromic anaemia, detected during a routine ante-natal check was investigated. The investigation revealed a double heterozygous haemoglobinopathy in a Caucasian family with no known Mediterranean ancestors for at least four generations. The abnormality was Hb D (Punjab) β Thalassaemia minor.

Case Report

In October 1979, Mrs K. A., a 33-year-old Caucasian, presented for a routine ante-natal screen and was found to have a haemoglobin of 87 g/l. The blood film showed a mild microcytic hypochromic anaemia with features of compensation. In December 1979, with the haemoglobin at 94 g/l, further investigations were carried out, including a screen for Thalassaemia. During a previous pregnancy, the presence of a mild anaemia was noted and attributed to β Thalassaemia minor as Mrs K. A. has a sister known to have Thalassaemia.

Electrophoresis of the red cell lysates on cellulose acetate at pH 8.6 revealed the presence of Hb A_2 Hb F and a dense band in the S/D region. Subsequent quantitation of these bands was done (Table 1). The raised levels of Hb A_2 and Hb F provided a partial diagnosis of β Thalassaemia trait.

Table 1

Hb A_2	**4.4%**	**(Normal 1.8-3.3%)**
Hb F	**3.8%**	**(Normal 0-0.8%)**
Hb S/D	**approx. 90%**	

The presence of Hb D was confirmed by negative sickling tests, by electrophoresis on citrate agar at pH 6.5 and later by peptide mapping (finger-printing) was found to be compatible with Hb D (Punjab). This lead to the conclusion of a combination of β Thalassaemia with HbD.

Because of the unusual nature of the haemoglobinopathy a family study was carried out, the results of which are presented (Figure 1).

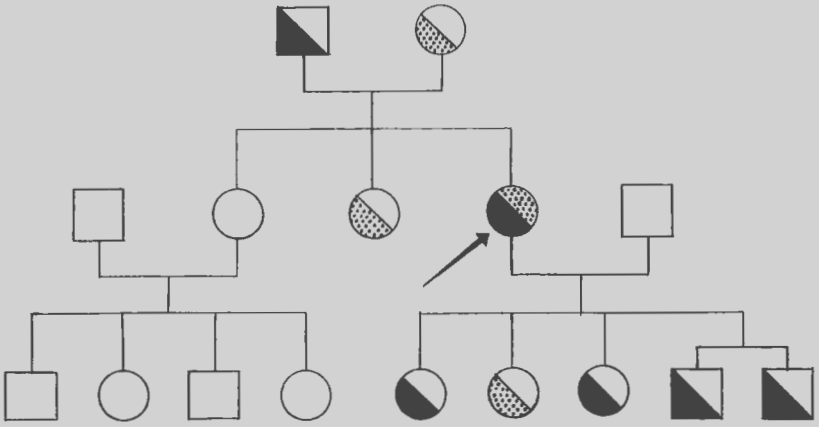

Figure 1.—Family tree of Mrs K.A.

Hb D Punjab

β Thalassaemia minor

Discussion

Since the initial work of Itano (1951)[1] on Hb D, numerous cases and studies have been reported showing that there are several variants of Hb D (Williams *et al.*)[2] and that since the first description of the haemoglobin in Los Angeles (known as Hb Los Angeles) there has been several alternative names for what is now known as Hb D Punjab, all of which have the same peptide map (α_2 β_2 121 Glu→Gln). Population studies have shown that approximately 3% of Punjab Indians have the haemoglobinopathy and that in other groups such as Caucasians the occurrence is sporadic.

The combination of β Thalassaemia with other haemoglobins such as Hb S and Hb C have been recorded previously as has the combination of β Thalassaemia and Hb D (Bird *et al.* 1956)[3]. Hence this case is not the first reported. But what does make this of interest is the occurrence of the double heterozygous abnormality in a Caucasian family, a family with no known Mediterranean ancestors for at least four generations.

This case has not been presented to dazzle anyone with a brilliant new discovery or to present some strange abnormal finding. Rather it should be taken as an encouragement to technologists in all laboratories, but particularly the small and medium sized, that rarities, oddities and unusual findings can be found in areas outside the main centres, if one keeps alert and on the look out for them.

Acknowledgments

I wish to thank Mr I. Bardsley of the Hawera Laboratory for referring this case to me and Professor R. W. Carrell for the technical assistance outside the scope of this medium sized laboratory.

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Haptoglobin Genotyping by Acrylamide Gel Electrophoresis in a New Zealand Population

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Received for publication, September 1980

Introduction

Serum haptoglobin, an alpha$_2$-globulin capable of binding haemoglobin, was first found to exhibit genetic polymorphism by Smithies (1955)[1], who used vertical starch gel electrophoresis (SGE) and demonstrated three common genotypes, namely Hp 1-1, Hp 2-1, and Hp 2-2, which were the products of two genes, Hp^1 and Hp^2.

The starch gel technique was subsequently developed at the Auckland Blood Transfusion Centre for use in family and population studies and was later used in medico-legal work. In 1972 an electrophoretic system was developed using the polyacrylamide gel electrophoresis (PAGE) method of Reid and Bieleski (1968)[2].

This report details this latter method as adapted to haptoglobin typing, and documents haptoglobin gene frequencies found in a New Zealand population.

Reagents and Equipment

1. Recrystallised acrylamide, recrystallised bisacrylamide and tetramethylenediamine (TEMED) were obtained from Eastman-Kodak. Other chemicals were as supplied by British Drug Houses (BDH).
2. A constant voltage electrophoresis power pack (Shandon), voltage range 0-400 volts, was utilised.
3. A water tight plasticine sealed glass mould built up from two glass plates (15 cm × 15 cm × 0.5 cm) clamped together but held 1 mm apart was constructed. A 10 fingered rubber slot former (used for the preparation of discrete sample chambers) was placed between the glass plates at one end. The inner glass plate had an oblong aperture near the top, through this, the acrylamide solution was poured into the mould for polymerisation.

Through this aperture also the electrode buffer has access to the gel during electrophoresis.

4. An electrophoretic assembly was used to which the glass moulded polyacrylamide gel could be attached vertically, with the anode and cathode buffer chambers being at the top and bottom of the gel slab, respectively. Further technical details are noted by Reid and Bieleski (1968)[2].

Method

a. To 25 ml of a 7.2% monoacrylamide solution containing 0.2% bis and 1.25% sucrose in 0.4 M tris—HCl buffer pH 9.0, 0.2 ml of 20% TEMED and 0.5 ml of 4% dipotassium per sulphate ($K_2S_2O_8$) was added, and the solution degassed for 20-30 seconds with constant mixing. This solution was then poured into the plasticine sealed glass mould, which had previously been filled and emptied with water to exclude all trapped air bubbles. The mould was kept free of disturbance until polymerisation was complete. After careful removal of the sample former, 15 ul of test sera was introduced into each chamber.

b. 1. *Haemoglobin solution*
Packed red cells were washed three times in saline. An equal volume of distilled water was then added to the packed cells, mixed, a half volume of toluene added, again mixed and centrifuged at 3,000 rpm for 30 minutes. The clear haemolysate layer was pipetted off and diluted 1/5 in distilled water for use.

2. *Treatment of test serum*
0.01 ml of haemoglobin solution was added to 0.05 of test serum and mixed. 50 mgm of sucrose crystals was added to this solution and allowed to stand for 10 minutes, followed by mixing. The sucrose was added to increase the viscosity of the serum sample, allowing it to layer evenly beneath the buffer in the well.

c. To allow the sample to enter the gel, electrophoresis was carried out at a constant voltage, initially at 60 volts (15mA) for 15 minutes and then finally at 120 volts (25mA) for four to five hours, without cooling. The acrylamide gel slab was run in a vertical position, with 0.2M glycine—0.025M tris pH 8.6, as the electrode buffer.

d. After electrophoresis the plates were parted and the gel stained. 2 ml of orthotolidine plus 0.5 ml of 20 volume hydrogen peroxide was freshly made up, mixed and poured over the gel. The solution was spread back and forth over the gel surface with a pipette, for 1-2 minutes. The excess stain was washed off with water and the results read. As haemoglobin and its iron-containing derivatives, all have peroxidase activity, oxygen is liberated from hydrogen peroxide and reacts with the orthotolidine to give a blue/green colouration.

Orthotolidine was made up as a 4% solution in ethanol. It was dissolved by heating then diluted ½ with glacial acetic acid which has been previously diluted ½ in distilled water.

Data for analysis of haptoglobin gene frequencies were obtained from accumulated paternity testing results.

In most instances, the ethnic origin of the persons involved was not available and a simple allocation into European and non-European was made. The latter group was known to contain a high proportion of Polynesians.

Statistical calculations of gene frequencies were calculated using the Hardy-Weinberg binomial equation and significance was determined using Student's "t" distribution in a double tailed test.

Table 1

Serum Haptoglobin Frequencies

		GENEOTYPE FREQUENCY (%)			*GENE FREQUENCY*	
No. Tested	Race	1-1	2-1	2-2	Hp^1	Hp^2
1577	Mixed N.Z. population	19.85	49.21	30.94	0.4445	0.5555
264	N.Z. Non-Europeans	37.88	47.35	14.77	0.6155	0.3845
295	N.Z. Europeans	13.56	46.78	39.66	0.3695	0.6305

Significance between non-Europeans and European Hp^1 gene frequencies was $t = 5.99$; $p < .0001$.

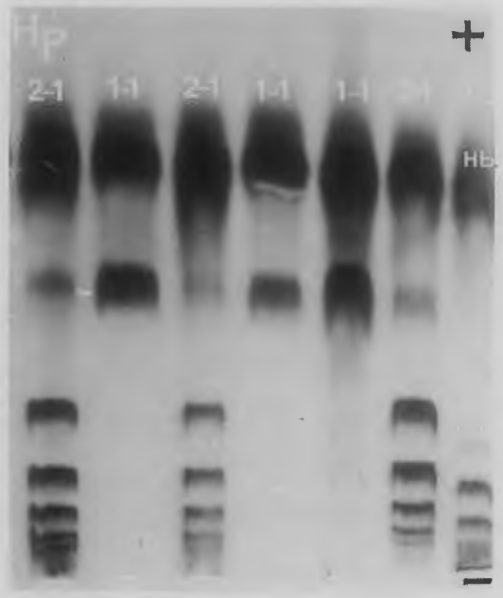

Figure 1.

Results

Typical results are seen in Figure 1.

Hp 1-1 migrates as a single band on electrophoresis but Hp 2-1 and Hp 2-2 migrate as a series of bands. The multi bands of Hp 2-1 and Hp 2-2 are made up of polymers of a monomeric unit of that genetic type. These polymers differing in molecular weight, move distances related logarithmically to their molecular weight. Hp 2-2 does not have the 1-1 band and the multi bands are slightly displaced compared to the Hp 2-1 multi bands.

When the haemoglobin added to the serum is not at optimal concentration to fully saturate all haptoglobin present, some intermediate complexes result and these migrate further than fully saturated Hp Hb complexes. The pattern is then one of multi bands, however these are easily differentiated from a true abnormality, by the absence of free haemgolobin, which is usually present in a region ahead of the Hp 1-1 band.

Absence of haptoglobins whether due to intravascular haemolysis or as in newborn infants, results in the type Hp 0-0, characterised by the absence of haptoglobin bands. Only a heavy band of free haemoglobin will be present after electrophoresis.

Results of testing for haptoglobin types are noted in Table 1. Significant differences are seen between European and non-European gene frequencies for Hp^1 and Hp^2, with a lower frequency of Hp^2 in non-Europeans.

Discussion

Acrylamide has a molecular weight of 71.08 Daltons. Polymerisation of acrylamide monomers is achieved by means of a cross-linker, a catalyst and an initiator. The cross-linker in the test system, is methylenebisacrylamide (bis), the catalyst is dipotassium persulphate ($K_2S_2O_8$), and the initiator, tetramethylenediamine (TEMED). The rate of polymerisation is controlled by the TEMED concentration, with increasing amounts of TEMED accelerating the polymerisation rate. The polymerised acrylamide or polyacrylamide forms an irreversible gel which is used for electrophoresis.

Acrylamide gel, like starch gel, is a molecular sieve as well as being a support medium in which protein electrophoresis can be carried out. However, unlike starch gel, the pore size of acrylamide gels can be varied directly by variations in gel concentration. Increases in gel concentration produce a decrease in pore size with a consequent exclusion from the gel during electrophoresis of molecules of decreasing size.

The acrylamide gel method has advantages over starch gel and these include ease of preparation of gels, a faster running time, a transparent gel, and because of the controlled pore size a much sharper definition of electrophoretic bands. The commercially available electrophoretic kitsets for polyacrylamide gel slab techniques, although usually of excellent quality, are expensive. Maintenance of our cheap locally made components has been minimal and the electrophoresis manifolds have not had to be replaced for eight years. The technique developed has been used routinely for haptoglobin genotyping for paternity dispute work, and has proved to be reliable and reproducible. The method will detect haptoglobin variants but their frequency must be low in a New Zealand population as only one variant has been detected in testing over eight thousand sera. Gene frequencies using this method are of some interest. There is a statistically significant difference in the distribution of haptoglobin types between the European and non-European groups. The non-European gene frequency of Hp^1 of 0.6155 is similar to that found by Douglas and Staveley (1960)[3] in Samoans (0.59) and Tongans (0.60).

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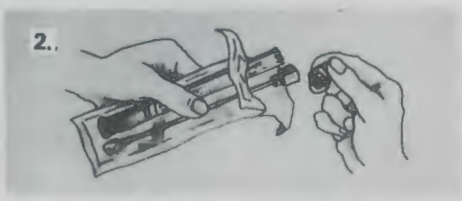

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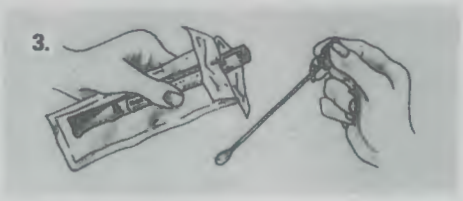

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Likewise the European Hp[1] gene frequency of 0.3682 is similar to that found in an English population (0.41). Allison *et al.* (1958)[4]. The frequencies for the mixed New Zealand population 0.4455) which is predominantly European, thus reflects the non-European content.

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Evaluation of an Enzymatic Amylase Kit

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Received for publication, September 1980

Summary

The new General Diagnostics' amylase method has been evaluated using the IL Multistat III centrifugal analyzer. Linearity extends up to 1000 U/litre. Day-to-day precision studies resulted in coefficients of variation of 6.8 to 2.1% over the range 25-340 U/litre. There was essentially no interference by icterus, haemolysis, lipaemia or glucose. Pyruvate at concentrations of greater than 0.5 mmol/litre interfered with the method. Activation of urinary amylase activity by albumin could not be demonstrated with this procedure. The reference range for serum is 11-70 U/litre.

Introduction

Measurement of serum and urinary α-amylase activity aids in the differential diagnosis of acute abdominal pain. Historically, saccharogenic, odometric, viscometric, turbidimetric and chromogenic or dye-starch methods have been used to determine amylase activity (Kachmar and Moss, 1976)[5]. Recent approaches to amylase estimation have centred on the use of water-soluble substrates with enzymatic coupling of the rate of substrate hydrolysis to the reduction of NAD^+. An enzyme-coupled procedure, based on the work of Pierre *et al.* (1976)[10] is used in a reagent system manufactured by General Diagnostics. The enzymatic reaction sequence is as follows:

$$\text{Maltotetraose} + H_2O \xrightarrow{\alpha\text{-amylase}} 2\ \text{maltose}$$

$$\text{Maltose} + \text{phosphate} \xrightarrow[\text{phosphorylase}]{\text{maltose}} \text{glucose} + \beta\text{-glucose-1-phosphate}$$

$$\beta\text{-Glucose-1-phosphate} \xrightarrow[\text{mutase}]{\text{phosphogluco-}} \text{glucose-6-phosphate}$$

$$\text{Glucose-6-phosphate} + NAD^+ \xrightarrow[\text{dehydrogenase}]{\text{glucose-6-phosphate}} \text{6-phosphoglucon} + NADH + H^+$$

For some years this laboratory has been using General Diagnostics' colorimetric Dyamyl-L amylase method. In late 1979 their enzymatic amylase method became available in New Zealand. General Diagnostics' new amylase method has been assessed in this laboratory; the results of the assessment are presented in this paper.

Materials and Methods

Reagents

The Chemstrate α-amylase reagent kit (Product number 8007, General Diagnostics, Division of Warner-Lambert Co.) contains 20 × 6.5 ml vials and currently costs $103.00. To compensate for dilution of the reagent by the loader, the vials were reconstituted with 5.2 ml of distilled water. The reagent was stored at 4°C. Additional reagents were: glucose, "Pronalys" (May and

Baker); lithium pyruvate monohydrate (BDH Biochemicals); human albumin, crystallised and lyophilized (Sigma Chemical Co.); bovine serum albumin (Armour Pharmaceutical Co.); Preciset cholesterol standards (Boehringer Mannheim); cholesterol reagent (Gilford Diagnostics); triglyceride reagent and lactate dehydrogenase reagent (Instrumentation Laboratory); glucose oxidase reagent and glucose standard (J. T. Baker Diagnostics).

Control sera and specimens

Versatol-E and Versatol-EN (General Diagnostics) were used as daily controls. Monitrol I and Monitrol II (Dade), Sigma 2E Enzyme Control (Sigma) and Ortho Elevated Bilirubin Control (Ortho Diagnostics) were used in kinetic, interference and reagent stability studies. Serum and urine specimens were obtained from laboratory personnel and hospital patients. Specimens were stored at 4°C and analysed within two days. Serum and urine pools were prepared from patients' specimens (supplemented with human saliva or fistula fluid when elevated amylase activity was required), aliquoted and stored frozen at −15°C.

Procedures

An IL Multistat III microcentrifugal analyser (Instrumentation Laboratory) was used for amylase measurements. Cholesterol, triglyceride and LDH levels were estimated on the Multistat by standard techniques. Glucose concentrations were determined with a Beckman Glucose Analyzer 2 (Beckman Instruments). Haemoglobin concentrations were estimated with a Unicam SP 800 recording spectrophotometer (Pye Unicam Instruments) according to Shinowara (1954)[11]. Urine protein was qualitatively assessed with Albustix (Ames, Division of Miles Laboratories) and quantified by turbidimetry (Meulemans, 1960)[8].

Table I details the Multistat settings appropriate for the amylase essay. Activity is measured at 30°C by monitoring the rate of NADH production after a lag period of 5 min. One unit of activity is defined as that amount of enzyme that in 1 min produces 1 μmol of NADH under the conditions defined. The factor is derived as follows:

Amylase, U/litre = $(\Delta A/\text{min} \times V_t \times 1000)/(6.22 \times 0.5 \times V_s)$ where $\Delta A/\text{min}$ is the change in absorbance per minute, 1000 converts U/ml to U/litre, V_t is the total assay volume (0.210 ml), 6.22 is the millimolar absorptivity of NADH at 340 nm, 0.5 is the pathlength (cm) and V_s is the sample volume (0.010 ml).

Table I

Settings for Amylase Assay

Loader Module Instructions
Sample volume, 10
Total sample volume, 40
Reagent volume, 64.0
Total reagent volume, 68.0
2nd reagent volume, 00
2nd reagent switch, off
Reference cuvette switch, diluent

Analyzer Module Instructions
Program 23 (General Enzyme), Enzymes Tape with the parameters:
Delay interval, 5 min 0s
Data time interval, 0 min 20s
Number of data points, 12
Filter code, 1 (340 nm)
Start mode, 0.001
Max. delta absorbance, 1.0
Temperature, 1 (30°C)
Factor, 6752

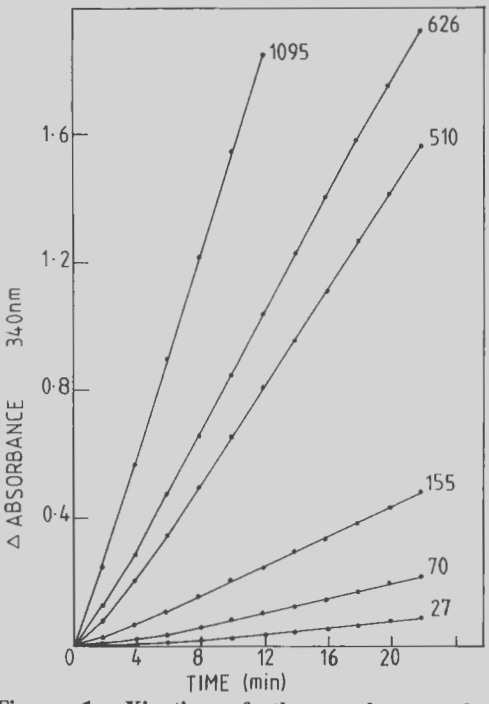

Figure 1.—Kinetics of the amylase method demonstrating the lag phase. Numbers associated with lines are the amylase activities, U/litre.

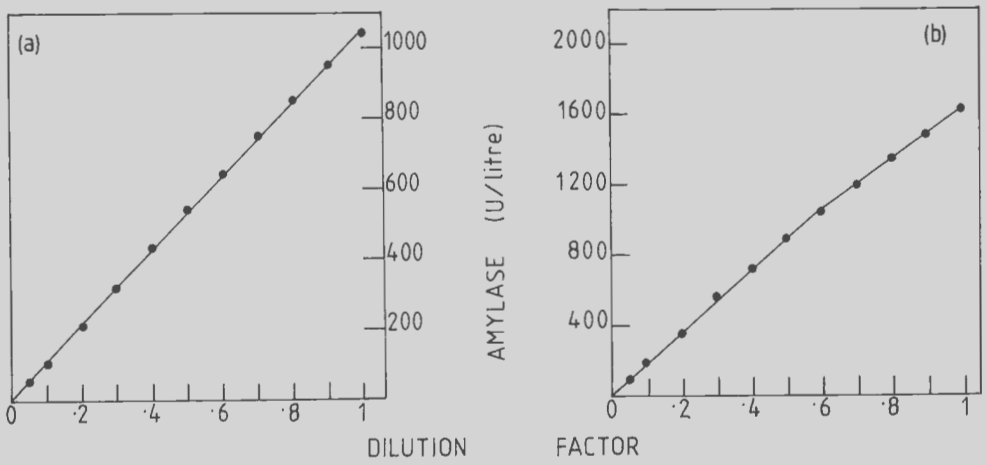

Figure 2.—Linearity of the amylase method:
(a) dilutions of human fistula fluid
(b) dilutions of serum supplemented with human saliva.

Results and Discussion

Kinetics of substrate hydrolysis

Reaction-rate curves of a group of samples covering a wide range of amylase activities are shown in Figure 1. Zero-order kinetics are established after a lag period of about 4 minutes. The manufacturer recommends an initial absorbance reading at 5 min at 37°C. Hence, this recommendation is also valid for an assay temperature of 30°C.

Linearity

Duplicate dilutions of human fistula fluid were prepared in physiological saline (145 mmol/litre NaCl) and analysed for amylase activity. The mean activity was plotted versus the dilution factor (Figure 2a). Linear regression analysis gave r = 0.9997 and intercept = 2.1 U/litre with a standard error of estimate (S_y) of 8.4 U/litre. Figure 2b shows a similar activity-dilution curve for a serum sample supplemented with human saliva. These plots indicate that the assay is linear up to 1000 U/litre.

Imprecision

Within-rotor imprecision was determined on serum pools at low, normal and high amylase levels. Day-to-day imprecision was assessed on both serum and urine samples. Table II summarises the results and shows that the analytical variation is dependent on the amylase activity of

Table II

Imprecision and Error Estimates

Imprecision	n	mean, U/litre	SD, U/litre	CV, %
Serum				
Within-rotor	17	15.4	1.48	9.6
	17	67.4	1.51	2.2
	17	254.2	3.92	1.5
Day-to-day	29	25.6	1.74	6.8
	29	64.0	2.69	4.2
	29	142.6	4.58	3.2
Urine				
Day-to-day	28	7.8	2.41	30.9
	28	106.6	3.13	2.9
	28	337.3	7.03	2.1

Random Error Estimates

	Serum	Urine
Allowable error, U/litre	14	60
Clinical decision level, U/litre	70	~300
Mean, U/litre	64.0	337.3
SD, U/litre	2.69	7.0
RE_u, U/litre	6.9	18.2
RE_l, U/litre	4.4	11.5

the sample. This suggests that instrument sensitivity is limiting at low amylase levels. Acceptability of a method's performance is based on the clinical significance rather than the statistical significance of errors (Westgard *et al.*, 1974)[13]. Accordingly, upper and lower confidence limits (95%) of the random error were calculated and compared with the allowable error. For amylase, Tonks (1968)[12] recommended a maximum allowable error of 20% of the measured value. Amylase activity at clinically significant decision levels (approximately upper limit of normal) were used in defining allowable error. Since the defined allowable errors are greater than the upper limits of random error, the (im)precision of the method is acceptable.

Reagent stability

Five serum pools and five commercial control sera ranging in amylase activity from 20-473 U/litre were analysed with freshly prepared reagent and subsequently with reagent that had been maintained at room temperature (20-22°C), 4°C and −15°C. Sufficient reagent (4 vials) for the entire experiment was reconstituted at the same time. The contents of the vials were combined, aliquoted into equal volumes and stored appropriately. All the serum samples were stored at 4°C between experiments. The data (Table III) confirms the manufacturer's specified reagent stability (24h at 2-8°C; 5h at room temperature). This excellent stability virtually eliminates waste and the reagent is particularly suited for "on-call" and "urgent" situations.

Table III

Reagent Stability

Reagent storage temperature, °C	Reagent storage time, h: 0	4	8	24	32	48
	Mean amylase activity, U/litre					
20-22	139	136	140	—	—	—
4	—	135	138	139	138	134
-15	—	—	—	131	—	—

10 samples were analysed with reagent that had been stored as specified above. Mean activities were calculated.

Table IV

Interference Studies

Sample composition	Amylase Activity, U/litre	Intercept, U/litre	r	S_y, U/litre
Bilirubin, 240 μmol/litre	364	4.9	0.9998	2.5
Haemoglobin, 8 g/litre	293	4.2	0.9995	3.5
Cholesterol, 14.8 mmol/litre Triglyceride, 31 mmol/litre	355	−3.5	0.9987	5.9

Activity-dilution plots of the three samples were subjected to linear regression analysis.

Effect of bilirubin, haemoglobin and lipaemia

On the basis of the results in Table IV the assay was judged to be free from interference by bilirubin, haemoglobin and lipaemia. Sigma 2E enzyme control vials were reconstituted with 5 ml of haemolysed or lipaemic serum pools. For elevated bilirubin, a vial of Ortho Elevated Bilirubin Control was reconstituted with 4 ml of normal serum pool; this was then used to reconstitute a vial of Sigma 2E. Activity-dilution plots of these sera were analysed by linear regression.

Effect of glucose

Glucose is a product of the reaction sequence. Conceivably, glucose might interfere with the assay by feedback inhibition of maltose phosphorylase. Glucose solutions (0.1 ml) were added to aliquots (0.9 ml) of serum with normal, raised and grossly elevated amylase activities.

Table V shows that the concentration at which glucose interfered was dependent on the amylase activity. Clinically, however, glucose interference is not significant.

Table V
Glucose Interference

Added glucose, mmol/litre	Amylase activity, U/litre Sample 1	Sample 2	Sample 3
0	23	345	1264
5.5	19	345	1270
11	23	342	1263
28	22	342	1253
56	23	343	1234
83	24	335	1212
110	25	329	1193

Endogenous glucose concentrations of samples were 4.8, 4.6, and 4.9 mmol/l respectively.

Pyruvate interference

Hanson and Yasmineh (1978, 1979)[2, 3] showed that with the Beckman enzymatic amylase methods elevated endogenous pyruvate interfered by reacting with the NADH produced to give lactate and NAD^+, thus reducing the increase in absorbance at 340 nm. This reaction was catalysed by LDH present as a contaminant in the kits. General Diagnostics Chem-Strate reagent was tested for LDH activity. Levels of 64 ± 5 U/litre were found, considerably less than the 4000 U/litre present in the Beckman reagents. Pyruvate solutions (0.1 ml) were added to aliquots (0.9 ml) of serum or urine. The amylase activity of these mixtures was then determined and the results are shown in Table VI. In line with the results of Hanson and Yasmineh (1979), pyruvate interference was inversely related to amylase activity. Generally, pyruvate interference is not significant until the pyruvate concentration exceeds 0.5 mmol/litre of sample (approximately seven-fold the upper limit of normal). This finding is in agreement with Hanson and Yasmineh (1979) despite the much lower LDH activity of the Chem-Strate reagent.

Table VI

Pyruvate Interference

Added pyruvate, mmol/litre	Amylase activity, U/litre Sample 1	Sample 2	Sample 3
0	33	123	202
0.02	32	124	206
0.05	34	122	210
0.1	34	122	205
0.2	33	120	195
0.5	33	121	200
1.0	1	106	197
1.5	1	24	205
2.0	2	2	75

Effect of albumin on urinary amylase

Urinary protein has been shown to have had an activating effect on urinary amylase when amylase activity was determined by chromogenic (O'Donnell and McGeeney, 1974)[9] and iodometric (Levitt *et al.*, 1977)[7] techniques. It is generally accepted that saccharogenic methods, thus enzyme-coupled procedures, are free from this effect. Recently, however, Garber and Carey (1978)[1] observed protein activation of urinary amylase with the Du Pont *aca* procedure. In this procedure maltotriose and maltose, produced from amylase action on maltopentaose, are hydrolyzed to glucose by α-glucosidase and ultimately the glucose is measured kinetically by the hexokinase/glucose-6-phosphate dehydrogenase couple.

In this present work activation of urinary amylase by protein or albumin could not be demonstrated. An activity-dilution curve (in 145 mmol/litre NaCl) of urine containing protein (3.3 g/litre) and amylase (78 U/litre) was a straight line. Linear regression gave r = 0.9989, intercept = −0.5 U/litre and $S_{y.x}$ = 1.1 U/litre. The possible enhancement of added protein was tested by

Table VII

Effect of Albumin on Urinary Amylase

Albumin, g/L	Amylase Activity, U/litre Sample 1	Sample 2
0	100	105
0.5	98	106
1.0	98	105
2.0	99	104
5.0	96	103
10	97	103
25	98	102
50	96	103

Samples 1 and 2 were from the same urine specimen. Sample 1 was supplemented with human albumin and sample 2 with bovine serum albumin.

an experiment in which amylase activity was measured as a function of albumin concentration. Aliquots of an early morning urine specimen, devoid of protein, was supplemented with human albumin or bovine serum albumin to a final concentration of 50 g/litre. The albumin was then diluted out with the original urine specimen. Amylase activity was unaffected by albumin (Table VII). This finding is important in view of the increasing demand for the amylase/creatinine clearance ratio and the influence of the amylase method on the ratio (Levitt *et al.*, 1977; Levitt and Ellis, 1979).[7, 6].

Reference ranges

Normal serum values for amylase activity were determined from laboratory personnel (8 males, 16 females) and from hospital patients (41 males, 49 females). The mean activities and SDs for the two groups were: mean = 36 and 34 U/litre; SD = 14 and 16 U/litre respectively. These data were combined and the frequency distribution is shown in Figure 3a. The distribution is non-gaussian. Accordingly, the percentile technique (Henry and Reed, 1974)[4] was applied resulting in a reference range of 11-70 U/litre.

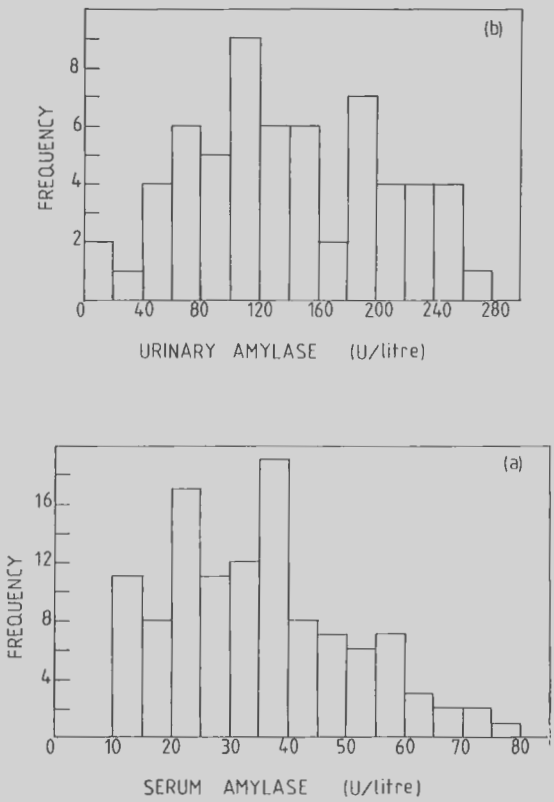

Figure 3.—Amylase reference range data:
(a) Histogram of serum amylase activity
(b) Histogram of urinary amylase activity.

Figure 3b shows the frequency distribution of urinary amylase activity in random and early morning collections from laboratory staff and their families (19 males, 42 females). A reference interval of 20-260 U/litre was obtained by the above method. This narrow range reflects the normal hydration status of the subjects. Urinary amylase levels of up to 460 and less than 10 U/litre have been obtained from normal subjects with fluid restriction and induced diuresis respectively (investigation in progress).

Conclusion

This method has been in routine use for six months. The procedure is reliable and convenient. Automation of amylase determinations has eliminated the accurate timing requirements and centrifugation steps of the manual Dyamyl-L method. Accordingly, the method has proved popular with "shift" and "on-call" staff. Cost, however, would prohibit the use of Chem-Strate reagent in a manual mode.

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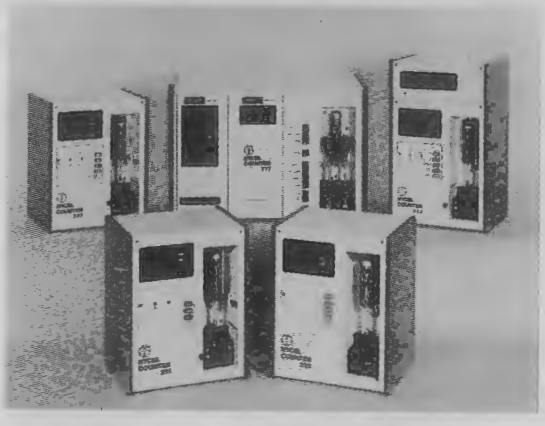

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Gc Globulin Types in a New Zealand Population

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Received for publication, November 1980

Summary

The immunoelectrophoretic method for the genotyping of Group-specific component (Gc) globulin is described. Results using the test revealed a significant difference in Gc gene frequencies between a European and Polynesian population. The method is reliable and accurate and is useful for paternity test investigations and in population genetic studies.

Introduction

Group-specific component (Gc) globulin, which is an alpha$_2$-globulin synthesised in the liver, functions as a carrier protein for vitamin D[1]. Genetic variation of Gc globulins was first noted in 1959 by Hirschfeld (1959)[2] who differentiated the various phenotypes by utilising an immunoelectrophoretic method. Three phenotypes were revealed, namely Gc 1-1, Gc 2-1 and Gc 2-2, which are controlled by two allelic genes, Gc^1 and Gc^2.

The immunoelectrophoretic method, which was originally described by Grabar and Williams (1953)[7] is a two stage technique. This involves an electrophoretic separation of the serum proteins in an agar gel, followed by an immunodiffusion reaction against a precipitating anti-Gc.

This paper details our methodology and presents the gene frequencies found in a New Zealand population.

Method

Twelve ml of a 1% molten agar solution (purified Oxoid Agar) dissolved in 0.03M barbital buffer—0.0025M calcium lactate, pH8.6, was poured onto a 10 cm × 10 cm × 1 mm glass plate. The agar covered the plate completely and evenly. When the gel was set, four sets of patterns were cut out (see Figure 1), each pattern having two wells (1 mm diameter), equidistant from a central trough (1 mm width). The agar was removed from the wells and into these were applied the serum samples using fine capillary tubes. The sample volume was 3 μl of undiluted serum, with the last sample being used as a dye control by making a 1 in 3 dilution of 0.5% Coomassie Blue in the undiluted serum. All samples were tested in duplicate.

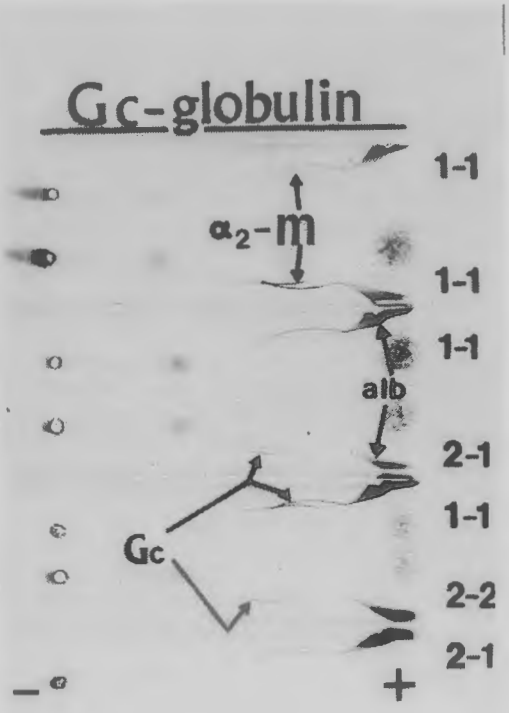

Figure 1.—Typical immunoelectrophoretic patterns demonstrating the alpha$_2$ macroglobulin (α2 –m), albumin (alb) precipitins, and the Gc globulin phenotypes (Gc). Electrophoresis from (–) to (+).

Two plates containing 16 samples were usually run together. Using 500 ml per electrode compartment of electrode buffer (0.05 M barbital—0.00125 M lactate, pH 8.6), the two gel plates were electrophoresed (with double thickness Whatman No. 1 filter paper wicks), at a constant voltage of 160 volts (5 volts/cm on the gel) for five to six hours at a current of 17-20 mA with fan cooling of the covered electrophoresis tank. Shandon power packs and Shandon electrophoresis tanks were used.

When the head of the dye had passed the end of the anti-serum trough, electrophoresis was discontinued. The agar was removed from the trough

cuts and the troughs filled with anti-Gc (Dakopatts 100 Gc) and left overnight in a moist chamber.

In the morning, the agar plates were dried. Initial moisture was extruded from the gel, by placing 12 thicknesses of filter paper (Whatman No. 1) over the gel area and then applying pressure for 20 minutes. After the filter paper was removed, complete drying was achieved using a hair dryer.

The plates were stained for 5-10 minutes in 0.5% Coomassie Blue (which had been dissolved in the decolourising solution consisting of a 50-10-50 volume mixture of ethanol, glacial acetic acid and water respectively). Decolourisation was performed until clear immuno-precipitation patterns had emerged. The plates were then dried and read. These plates were filed as permanent records.

Blood samples

Blood samples were obtained from two groups. Firstly, data was available on blood samples taken from 783 non-related adult males and females who presented themselves for paternity dispute investigations. The ethnic origin of the persons concerned in paternity disputes was noted in some paternity investigations, and from these data, information on 636 New Zealand European donors was obtained. The second group consisted of samples from random blood donors who were clearly identified as Pacific Islanders. These Pacific Islanders were predominantly Samoan with smaller numbers of Tongans and Niue Islanders (Table I).

Results

Typical results are seen in Figure 1. The Gc antiserum contains antibodies against alpha$_2$-macroglobulin, albumin, and Gc globulin. The first two antibodies form precipitin arcs with their respective proteins and these then act as reference markers whereby the Gc phenotypes can be differentiated. The Gc 1-1 is a fast moving single arc as compared to the Gc 2-2 which is a slower moving single arc. The Gc 2-1 is a continuous, double arc, precipitin line which appears as a "seagull" pattern.

Frequencies of phenotypes are seen in Table I. The New Zealand (mixed) group showed no difference in gene frequencies when compared to the results of a study by Cleve (1961)[3] on USA whites. However, when the Polynesian donor group was tested, a significant difference ($t = 2.05$; $2P < .05$) was shown between the Gc^1 gene frequencies when compared to the New Zealand European group.

Gc globulin typing was used in paternity dispute investigations and results from a recent review are outlined in Table II. The usefulness of Gc typing in excluding non-fathers compared well with other major blood group systems, i.e. Gc detected 16.7% of non-fathers while the ABO system detects 17.6% of non-fathers. Race *et al.* (1968)[4]. These data on Gc typing in paternity disputes are in accord with that reported by Sussman (1976)[6]. Furthermore, four possible non-fathers were detected, these persons not being excluded by any other of the blood group systems used e.g. ABO,

Table I

Gc Globulin Frequency

		Gc 1-1		Gc 2-1		Gc 2-2				
Group	No. Tested	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Gc^1	Gc^2	X^2
N.Z. (mixed)	783	403	401.94	316	318.12	64	62.94	.7165	.2835	0.035
%		51.47		40.36		8.17				
N.Z. European	636	334	331.98	251	255.04	51	48.98	.7225	.2775	0.159
%		52.52		39.46		8.02				
Polynesian *	215	89	89.86	100	98.27	26	26.87	**.6465	.3535	0.067
%		41.39**		46.51		12.09				

* These specimens were obtained from random Pacific Islander blood donors. The following numbers of Polynesian samples were available: Samoan 177, Niuean 15, Tongan 23. Numbers tested were too small to detect significant variations in gene frequency between Island groups.

** Significant difference ($2P < .05$) from New Zealand European group by double tailed students "t" test.

Table II

Gc Typing in Paternity Dispute Investigations

No. of samples tested	385	
No. of exclusions (all systems)	90	(23.4%)
No. with Gc exclusions	15	(16.7%)
No. with single Gc exclusions	4	

Rhesus, MNSs, Kell, Duffy (Fy^a), Kidd (Jk^a), Haptoglobin (Hp) and Phosphoglucomutase (PGM).

Discussion

Several technical points in the methodology should be noted. Firstly, the agar gels are thin and this results in less reagents i.e., agar, buffer, serum sample and anti-Gc, being used as compared with other methods. Secondly, the sample volume is critical, as excess sample results in blurring of the immunoprecipitate (i.e. prozone effect) while a small sample will not reveal Gc bands. Thirdly, electrophoresis of the serum proteins must be equidistant from the trough throughout, otherwise non-readable immunoprecipitin patterns will result. This can be best achieved by ensuring that the gel plates are lined up parallel to the electrical flow. Fourthly, the type of agar is important. This method has been standardised against the various technical variables with "Purified Oxoid Agar". If other purified agars are used, standardisation must be performed, as different agars have varying electroendosmotic properties. Finally, anti-Gc is commercially available from a number of commercial sources. Some anti-Gc's contain antibodies against many proteins and the resulting immunoprecipitins can produce a very confusing picture with Gc bands being difficult to identify. In our experience the "Dakopatts" antisera appears to have only antibodies against albumin, $alpha_2$ macroglobulin and Gc globulin, and thus the resulting patterns are clearly readable.

No data appear to be available on Gc frequencies in New Zealand although it might be expected that these would be similar to those of other Caucasian populations. The Polynesian proportion of the population does not appear to have significantly affected the total Gc frequency, probably because of the high rate of intermarriage between Polynesian and European. It is only when a more clearly defined population such as the Pacific Islander group is studied that a significant difference in gene frequencies can be detected. It is of interest to note that Polynesian Cook Islanders have a Gc^1 frequency similar to Caucasians. Kenrick (1967)[9]. Although there are at least 10 known Gc variants, Prokop *et al.* (1969)[8], none were detected in this study.

Recently Gc globulin genotyping has been accomplished by isoelectric focusing, providing further evidence of genetic variation within the system. Kuhnl *et al.* (1978)[5]. By this method, three further subtypes can be detected. Although the technique is expensive and requires high voltage electrophoretic equipment this method may increase the usefulness of Gc typing when applied to medico-legal problems.

The results in paternity dispute tests verify the usefulness of the method in this type of investigation. The absence of detectable variants indicates that substantial reliance can be placed on an exclusion based on Gc typing. Further population studies of Gc groups in differing ethnic populations are indicated.

Acknowledgment

The assistance of Dr D. G. Woodfield, Medical Director, Auckland Blood Transfusion Centre, in the preparation of this paper is gratefully acknowledged.

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A Study of the Imugard TF:IG500 Filter for the Production of Leucocyte Poor Blood

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Received for publication, December 1980

Summary

The Imugard TF:IG500 filter for the production of leucocyte poor blood is evaluated using haematological, biochemical and microbiological methods and compares favourably with our laboratory results of alternative methods for leucocyte poor blood production.

Introduction

Leucocyte antibodies are considered to be the principal cause of non-haemolytic transfusion reactions. Issitt *et al.* (1975)[7]. The majority of these are febrile in nature and not life threatening, but may impede recovery of the patient. AABB (1975)[2]. According to Issitt, it is possible that a reaction due to leucocyte antibodies could cause destruction of red cells because of complement activation. Patients who have had numerous transfusions, multiparous women and patients having had tissue or organ transplants often develop leucocyte antibodies. AABB (1975)[2].

In order to prevent reactions due to leucocyte antibodies, removal or at least a substantial reduction of leucocytes from the transfused blood is desirable.

Methods for the removal of leucocytes from whole blood are often time consuming and carry a risk of contamination but are technically feasible and clinically useful. AABB (1975)[2]. The method selected depends on the need of the patient and resources available to the Blood Bank. Abelson (1974)[1].

Before any method or technique is accepted for routine use, its reproducibility must be established by those who are to routinely use it. This process of validation must be thorough. If the technique has any inherent weaknesses, the test conditions should bring these to light. Techniques already in use should be compared with possible alternatives to ensure that, in the hands of all who are to use them, they are the most suitable, reliable and reproducible. ISBT (1978)[6].

As early as 1926 Fleming and Wright, [Kikugawa *et al.* (1978)[8]] described the absorption of leucocytes by cotton and filter paper. Since then several substances have been used to filter leucocytes out of whole blood. At present, the raw cotton of *Gossypium barbadense* seems to be the superior filling for filters. Kikugawa *et al.* (1978)[8].

Materials and methods

The Imugard TF:IG500 filter, manufactured by the Terumo Corporation, designed for the preparation of Leucocyte Poor Blood, was tested for consideration as the routine method for processing in this region. A suitable quality control programme was established and the following criteria carefully examined: percentage leucocyte removal, red cell recovery, the possibility of bacterial contamination, haematological and biochemical parameters, comparison with alternative methods.

Description of Filter

The filter is packed with raw cotton of *Gossypium barbadense* and is suitable for the preparation of leucocyte poor blood for clinical use. A unit of whole blood can be filtered within 30 minutes and aseptic technique must be used. Kikugawa *et al.* (1978)[8], see Diagram 1.

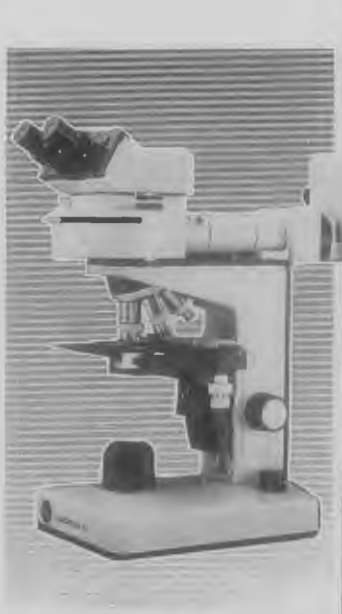
Methods of Leucocyte Removal

The following are methods currently available:

1. Saline washing. AABB (1975)[2].
2. Haemonetics and Continuous Flow. Haemonetics (1979)[5].
3. Nylon Filtration. AABB (1977)[3].
4. Inverted Centrifugation. AABB (1975), AABB (1977)[2, 3].
5. Frozen-Thawed Red Cells. AABB (1975)[2].
6. Dextran Sedimentation. AABB (1975)[2].
7. Double Dextran Sedimentation. Faed *et al.* (1978)[4].
8. Imugard Filtration. Kikugawa *et al.* (1978)[8].

For our complete programme, a total of 80 units of whole blood were tested. Several filters were tried using a variety of fresh and stored units of whole blood. The manufacturers state that the filter is intended for single use only, Terumo Corporation[9], however, using the same procedure, several units were put through a single filter.

The following tests were carried out on pre-filtration specimens collected aseptically from the



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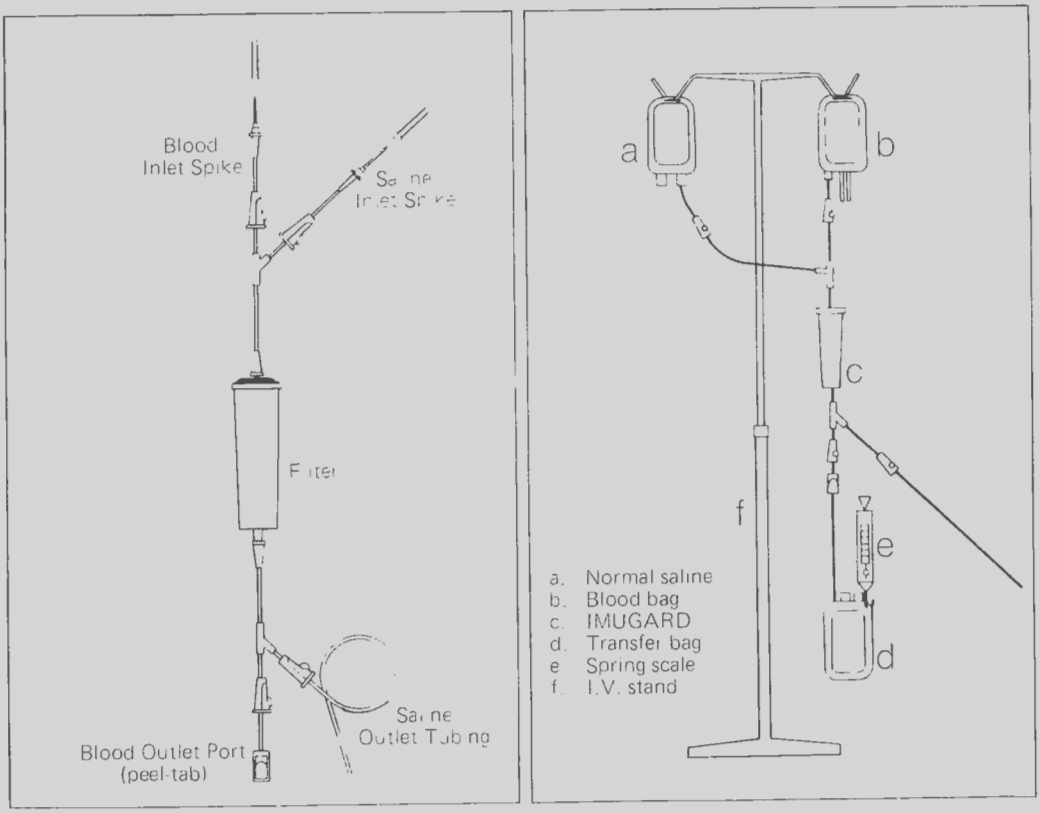

Figure 1

Table 1:

Comparison of Current Methods in our Laboratory

Method	% leucocyte removal	Red cell recovery	Haematological evaluation	Biochemical evaluation	Possibility of contamination	Time taken	Ease of performance	Comments
1	62	reasonable	poor	*	high	time consuming	fair	most plasma proteins removed
2				Not included in the study				
3	50-65	good	fair	*	high	time consuming	fair	lymphocytes are not removed
4	63	below average	good	good	low	20 minutes	good	blood 6 days old can be used
5	79	fairly good	*	*	high	*	*	methods not available as yet
6	68	70%	reasonable	reasonable	high	45 minutes	average	dextran is antigenic
7	81	70%	reasonable	reasonable	high	90 minutes	average	2.5% dextran used
8	88	very good	very good	very good	low	20-30 minutes	good	costs approximately $9-50

* These variables not available from this study

unit to be filtered and post-filtration specimens collected aseptically from the collection bag after mixing. Total leucocyte counts and red cell indices were performed on the Coulter S. Percentage differential counts on May Grunwald-Giemsa stained films. Urea and electrolytes peformed on the SMA 6/60, utilising colourimetric and flame photometric methods. Aerobic and anaerobic blood cultures were carried out on all pre- and post- specimens from all units filtered. All the above results were collated.

Results

Table 1: Comparison of current methodology in our Laboratory.

Table 2: Leucocyte counts: Pre- and Post-Imugard filtration.

Table 3: Leucocyte counts: 2 units through one Imugard filter.

Table 4: Haematological values: Pre- and Post-Imugard filtration.

Table 5: Differential Count: Pre- and Post-Imugard filtration.

Table 6: Biochemistry values: Pre- and Post-Imugard filtration.

Table 2:
Leucocyte Counts: Pre and Post Imugard Filtration

Unit	Pre white cell count $x10^9/\ell$	Post white cell count $x10^9/\ell$
1	4.6	0.4
2	4.1	0.1
3	5.9	0.8
4	4.0	0.2
5	7.3	1.1

Table 3:
Leucocyte Counts: 2 units through one Imugard Filter

Unit	Pre white cell count $x10^9/\ell$	Post white cell count $x10^9/\ell$	% leucocyte removal
1	4.1	0.1	98
2	4.6	0.4	91

Table 4: Haematological Values: Pre and Post Imugard Filteration

	WCC $x10^9/\ell$	RCC $x10^{12}/\ell$	Hb g/ℓ	PCV	MCV fl	MCH pg	MCHC g/ℓ
Pre	5.9	3.58	115	0.325	92	31.5	351
Post	0.8	3.46	115	0.315	91	31.0	351
Normal values	4.0–11.0	3.9–6.5	120–180	037–054	76–96	27–32	320–360

Table 5:

Differential Count: Pre and Post Imugard Filtration

	Metamyclocytes %	Neutrophils %	Lymphocytes %	Monocytes %
Pre	2	61	34	3
Post	0	60	40	0
Normal values	0	40–75	20–45	2–10

Table 6:

Biochemistry Values: Pre and Post Imugard Filtration

	Sodium mmol/ℓ	Potassium mmol/ℓ	Carbon Dioxide mmol/ℓ	Urea mmol/ℓ	Creatine mmol/ℓ
Pre	173	3.2	18	6.1	0.120
Post	169	2.9	16	5.7	0.110
Normal values	133–148	3.5–5.0	22 –32	2.6–6.8	0.05–0.10
2 Std. Dev.	3.0	0.2	4.0	1.0	0.026

Discussion

The instructions for use must be followed, although we found no pressure on the blood bag was necessary to complete filtration within 30 minutes.

The manufacturers state that 95-100% of leucocytes are removed from packed cells and 95% of red cells are recovered. Kikugawa *et al.* (1978)[8]. The 88% mean leucocytes removal was obtained using the Imugard filter. Running two fresh units through one filter also gave a comparable leucocyte removal. There was no appreciable change in the red cell indices and the urea and electrolyte parameters did not alter beyond the normal standard deviations for each of the methods used. The PCV could be adjusted with the addition of saline.

It was noted that the citrate phosphate dextrose solution, added to the unit of whole blood as an anticoagulant, raised the sodium levels and lowered the potassium levels.

No evidence of bacterial contamination in the blood cultures was observed.

Conclusion

Under test conditions in our laboratory using the Imugard filter, red cell recovery and leucocyte removal proved to be acceptable. With respect to red cell indices, urea and electrolyte parameters, no unexpected changes were observed. If manufacturer's instructions were adhered to, and sterile equipment utilised, no bacterial contamination occurred.

Although the manufacturers stated that the filter is for single unit use only, we found that two units, if for the same patient, could be filtered, the second immediately after the first, through the same filter with no appreciable change in the parameters.

The Imugard TF:IG500 filter appears to be a very effective method for routine use and could prove to be invaluable for use in small laboratories where the preparation of leucocyte poor blood is seldom undertaken. It is a quick, effective, simple and inexpensive method.

With current developmental work being carried out on filtration systems it is envisaged that the employment of an in line filter will be routine for the infusion of Leucocyte Poor Blood.

Acknowledgments

The authors wish to acknowledge the help of the New Zealand Blood Transfusion Services, Otago Region Blood Donor Centre, Haematology, Microbiology, Chemical Pathology and Immunohaematology, Dunedin Public Hospital, with special thanks to Les Milligan.

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A Useful Adaptation of the B-D Microtainer Capillary Collection System

G. T. Goodman, ANZIMLT
Pathology Department, Nelson Hospital

Our laboratory has been using the B-D Microtainer method for collecting haematology capillary specimens for the past nine months.

In our experience we have found that unless a very good flow of blood is obtained, the blood tends to accumulate in the Microtainer capillary collector nozzle and hinder the free flow of the blood into the Microtainer tube.

By inserting a heparinised capillary tube down the interior bore of the collector nozzle, blood collection is considerably enhanced. As 200-300 μl of blood is collected into the Microtainer tube, the effect of the heparin in the capillary tube on the blood appears to be negligible.

Inhibition of Pseudocholinesterase by Serum Separators

M. R. Lawson
Grade Technologist, Auckland Hospital

It is known that some plastics contain substances which inhibit pseudocholinesterase and it is recommended that all reagents be stored in glass bottles. Dietz *et al.* (1973).

When checking the standardisation of our semi-automated pseudocholinesterase method, an adaption of the method of Ellman (Dietz *et al.* 1973)[1] using propionylthyocholine as substrate it was discovered that random serum samples that had been stored for one week at 4°C in a Technicon Seraclear had no pseudocholinesterase activity at all.

To investigate this we obstained five brands of serum separators and collected blood from members of the laboratory staff. The levels of enzyme were measured after serum was separated without serum separators and after exposure to serum separators for times as detailed in the following table:

All samples from one serum separator were measured in one batch on Day 7.

Serum Separator Brand	No Serum Separator	Serum Separated with and Exposed to Serum Separators					
		0 hrs	6 hrs	1 day	2 days	3 days	7 days
Technicon Seraclear Cat. No. 510/4055/01. Supplied by Technicon.	4.8	1.0	0.2		None detected		
Evergreen Sera Separa No. 3193. Supplied by Roche Products.	4.5	4.5	4.6	4.5	4.5	4.6	4.9
Elkay EZEE Filter Separator 1273193025. Supplied by Medic DDS.	4.6	4.5	4.6	4.6	4.4	4.4	4.5
Glasrock Filter Sampler FS 216. Supplied by Biotek Supplies.	4.0	3.9	4.0	4.0	4.0	4.0	4.0
Unichem Filter Sampler Cat. No. P5190-4. Supplied by McGaw Ethicals.	4.7	4.7	4.8	4.8	4.9	4.8	48

From this it would appear that the Technicon seraclear causes considerable inhibition of pseudocholinesterase whereas the other brands do not. The filter portion of the seraclear caused more inhibition than the plastic tube. A serum with a pseudocholinesterase level of 6.3 units was exposed to the filter and plastic tube portions of a seraclear for two days; the resulting pseudocholinesterase levels were none detected and 4.1 units respectively.

Serum aspartate aminotransferase and alkaline phosphatase estimations were done on the sera that were exposed to the Technicon seraclears for one week but there was no change in these enzyme levels.

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Book Reviews

Manual of Clinical Immunology. Edited by Noel R. Rose and Herman Friedman. Published by the American Society for Microbiology, Washington D.C. 1980. Second Edition. 1105 pages, illustrated. Price US$25.00 (cloth), US$21.00 (flexible).

This manual represents a joint effort by the American Society for Microbiology and the American Association of Immunologists to provide medical scientists and physicians interested in clinical immunology with a guide to the rapidly growing field of laboratory immunology. It is directed mainly to laboratory personnel responsible for the performance of immunological tests in the clinical laboratory.

The methods are described simply but generally with sufficient detail to avoid referral to other sources. The techniques are those used by the authors with brief discussions of other approaches. Reactions devoted exclusively to the identification of micro-organisms are dealt with in companion volume "Manual of Clinical Microbiology."

This volume has been very thoroughly rewritten, many of the contributors (over 200) are new, there are two new sections: "General Methods and Procedures" and "Complement and Immune Complexes."

The authors submitted to a peer group review procedure and the quality of the book reflects this painstaking procedure. There are 145 chapters arranged in 13 sections "A" to "M".

A. General Methods and Procedures for Measuring Humoral and Cellular Factors. Twelve chapters, 84 pages. The topic is very thoroughly covered, indeed as might be expected different authors cover the same ground in some instances. Immunofixation, nephelometry, flow cytometric analysis and immune electron microscopy are some of the areas discussed. R. F. Ritchie in the introduction remarks on the difficulty of producing specific standard antisera or of obtaining information on how to do it. Consequently there is not a specific chapter on this subject. In the Editors' general introduction this problem is also alluded to and hope for its resolution expressed in the developing technique of monoclonal antibody production from hybridomas.

B. Tests for Humoral Components of the Immunological Response. Six chapters, 55 pages. This includes chapters on assessment of T-cell help and suppression of B-cell function and quantitative RIA for antibodies and immuno-globulin produced in vitro.

C. Complement and Immune Complexes. Five chapters, 30 pages.

There is a new chapter on C1-INH. The pros and cons are described and details of an enzymatic inhibition method based on the original article by Levy and Lepow. (A simplified version was published recently in J. clin. Pathol. (1980), 33, 167).

Three other chapters dwell on the detection of immune complexes and the first by Vincent Angello refers to the recent WHO comparative survey of different methods and the need for standardisation of methods and reagents to make sense. This author uses $C1_q$ and mRF inhibition RIA. These methods are described in detail. Two of his comments are worth repeating. 1: The practical clinical application of immune complex assay has still to be determined. The large scale clinical studies required to provide this information have not been performed because of the lack of a simple method. 2: Some low molecular weight

substances which are not immune complexes found in SLE and other diseases, may react with the $C1_q$ reagent. They do relate to the disease process and point to the value, even if empirical of this reagent.

D. Tests for Cellular Components of the Immunological Response. Sixteen chapters, 118 pages. Two new chapters appear in this large section, lymphocyte mitogenic factor and lymphocyte-mediated cytotoxicity.

E. Immunoassays. Sixteen chapters, 96 pages. This has been largely rewritten. Specific techniques for the thyroid, pituitary and adrenal hormones have been deleted but it provides newer techniques such as ELISA, RIA for myelin basic protein, acetyl choline receptor antibodies, thyroid stimulating antibodies (from the Immunopathology Department of Otago Medical School), prostaglandins and liver membrane lipoproteins.

F. Bacterial, Mycotic and Parasitic Immunology. Twenty-four chapters, 175 pages. Four new chapters, *Haemophilus influenzae* Type b capsular polysaccharide and anti-polysaccharide antibody assays, immune response to *Legionella pneumophila,* immune response to *Streptococcus pneumoniae,* C-reactive protein.

G. Virus, Rickettsial and Chlamydial Immunology. Seventeen chapters, 95 pages. Some revision and change of authors.

H. Immunohaematological Tests. Six chapters, 119 pages. New chapters: detection of neutrophil and platelet antibodies, human allotype detection by passive haemagglutination.

I. Laboratory Examination of Patients with Allergic and Immunodeficiency Diseases. Eight chapters, 59 pages. One new chapter on tests for immunological drug reactions.

J. Immunological Tests for Autoimmune Diseases. Ten chapters, 57 pages. Same topics, some change of authors.

K. Immunological Tests in Tumour Immunology. Five chapters, 34 pages. Two additional chapters: calcitonin in RIA (and its specific application in detecting familial medullary carcinoma of the thyroid), prostatic acid phosphatase. This presents the current outlook and details the immunological assays required to detect the early changes of prostatic carcinoma and to evaluate chemotherapy.

L. Immunological Tests in Transplantation Immunology. Twelve chapters, 132 pages. There are some changes of author in this section and a new chapter: cellular typing.

M. Licensure and Certification in Clinical Immunology. Seven chapters, 30 pages.

I reviewed the first edition in March 1977 and the same comments still hold. This is a very practical reference manual presenting basic information and attempting with considerable success to provide a compendium of the available techniques. I find it one of the most useful books on this subject. $21 seens remarkably cheap for a work of this size.

— R. D. Allan

Microbiology, Health and Hygiene. Justus A. Akinsanya, BSc (Hons) Lond. With a special contribution by: Malcolm J. W. Hughes, BSc (Hons) Lond, PhD (B'ham). Published by Macmillan Press Ltd, London and Basingstoke. Obtained from Macmillan Co. of New Zealand Ltd, 9 Goldfield, P.O. Box 33-570, Takapuna, Auckland 9, New Zealand. Soft cover, illustrated, 112 pages. Price NZ$15.00 retail.

This is a comprehensive, soft covered, text written specifically for health care workers in developing tropical regions. The style is concise and readable and stresses the practical application and problems facing workers in these regions. It adopts a basic practical approach to microbiology, health, hygiene and related paramedical disciplines. This text would provide a current knowledge for the laboratory technologist and other paramedical staff new to these regions and will assist them in providing a realistic, preventative and curative service for the patients.

L.M.M.

A Guide to Medical Entomology. Author: M. W. Service. Publisher: Macmillan Publishers London and Basingstoke. Obtained from: The Macmillan Co. of New Zealand, 9 Goldfield, P.O. Box 33-570, Takapuna, Auckland 9. Soft cover, 226 pages, illustrated. Price NZ$32.30 retail.

This textbook is one of the Macmillan International College Editions. It is well laid out with numerous clear line illustrations of each relevant arthropod at various stages of its development. The book consists of four basic introductory chapters to the arthropoda, 20 chapters each devoted to a particular group of pests and four chapters dealing with techniques.

The introductory chapters are informative and easily understood. They provide the basic information necessary to understand medical entomology but avoid unnecessary detail.

The central chapters provide the details necessary for the identification of particular pests, the life history, medical importance, and the

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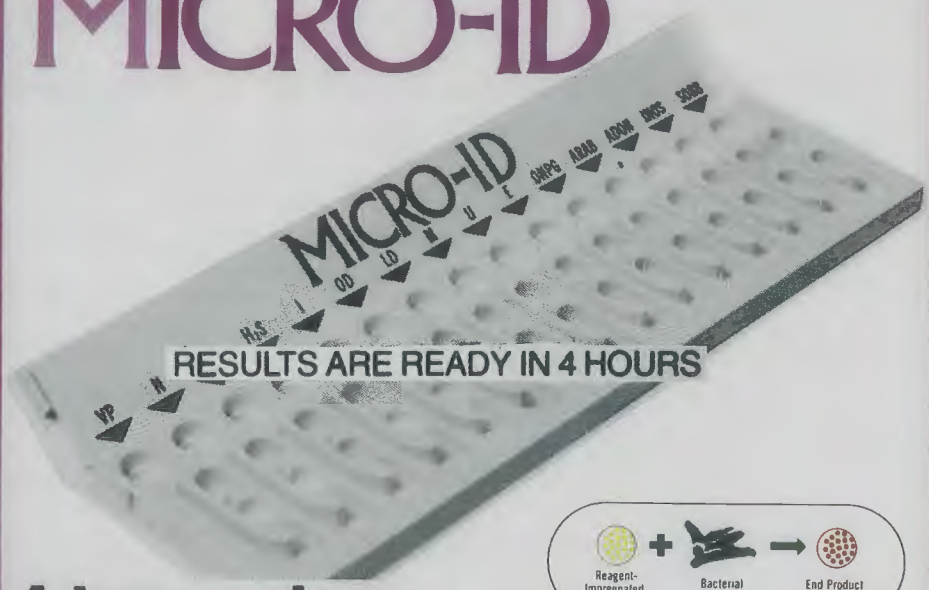

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measures necessary for their control. The final chapters cover the practical laboratory details of collection, dissection, storage of arthropods and the use and formulation of insecticides.

The author is head of the Entomology Department of the Liverpool School of Tropical Medicine and Hygiene and the illustrations are by S. N. McDermott of the Institute of Medical and Veterinary Science, Adelaide, Australia.

There is a comprehensive bibliography to each chapter and a good index.

I have only one criticism and that is the failure to mention the dangerous spiders of Australasia.

This book could be particularly recommended to those working in the field away from the more sophisticated reference books.

John Lucas

Biochemistry. A Case-Oriented Approach. Third Edition, 1980. Montgomery, Dryer, Conway and Spector: C. V. Mosby. From N. M. Peryer. $28.50.

This is a bulky book (over 700 closely packed pages) designed, so the authors tell us, to be used as the basis of a 14-week course in biochemistry. It is particularly slanted towards students of "health sciences". Each chapter contains a set of objectives, basic biochemical information, and a series of case presentations, each with a number of questions and references. Some of the cases are discussed in detail, a useful way of including a good deal of chemical pathology in what would otherwise be a very basic text. The final chapter consists of yet more cases, again discussed in greater detail.

This approach is an extremely interesting one. While the book is much too long and complex to be of use in teaching laboratory staff, it appears that it may be useful for medical students, combining as it does much of the basic, preclinical information with some rather superficial chemical pathology.

However, the book is not without its faults. It is extremely detailed, and it requires some diligence to read and pick out the salient points in each topic. The authors have apparently become converted to the S.I. and use the Units with an evangelical fervour, but have established numerous discrepancies in the process. Hemoglobin, albumin and total protein are measured, they say, in mmol/l or g/dl; pressures (even those of blood) are given in kPa, as well as mmHg; enzyme activities are reported in a bewildering variety of units, including the little-used katal.

There are also numerous "typos", some quite amusing (e.g., "buffer" is given as "butter" twice on one illustration). More serious, perhaps, are the debatable points in the case presentations and discussions. The methods used to diagnose PKU and galactosemia are obsolete, and there is no mention of screening programmes for those or other inborn errors of metabolism. Cystinosis and cystinuria are confused; Dextrostix are used in urine testing. BUN "rose" in a case of hepatic coma (it may well do, but is likely to be surprisingly low, given the state of the patient). As is usual in a book of this nature, the cases selected range from the very rare (Zellweger's syndrome) to the mundane; an unwary student might gain a very distorted view of the incidence of some diseases.

Since this book is now in its third edition, it obviously has its admirers and users; with a few reservations, I am prepared to join both groups.

Chris Lovell Smith

Theory and Practice of Histotechnology. Dezna C. Sheehan and Barbara R. Hrapchak. Second Edition, 1980. Published by the C. V. Mosby Company, St Louis. Four hundred and eighty-one pages, illustrated. Price $44.95. Supplied by N. M. Peryer of Christchurch, CPO Box 833.

This American book has been written mainly by two Education Co-ordinators in Histotechnology with contribution by specialists in such fields as Immunohistochemistry and Electron Microscopy.

The book begins with a detailed and up-to-date review of equipment, written in such a manner that one feels that the authors have personal experience of the instruments used, and are aware of the problems likely to be encountered in a routine laboratory. This air of practicality is present through nearly the whole volume.

Techniques for studying bone by decalcified and undecalcified methods are described in some detail and there is a section on Histomorphic analysis which may well encourage greater use of such an approach in evaluating disease processes. For technologists involved in the more classical staining methods, the mid section of the book is of particular value. There is a review of the theoretical aspects of staining which sheds light in an area where empiricism has often been the rule. This is followed by a comprehensive description of staining methods in which the theoretical principle and practical difficulties are given due regard.

The American origin of this book is not too obvious, the syntax and spelling do not cause hesitation in reading, however the absence of stains of British origin is sometimes noticeable, e.g. Lendrum's stains for fibrin could well be included.

This is a book for the 1980s and it is fitting that it should review enzyme techniques in some depth, and have a chapter on Immunohistochemistry explaining the theory and practice of Horse Radish Peroxidase methods as well as Immunofluorescence techniques.

Electron microscopy is covered in one chapter, adequate for the novice, but no substitute for specialist text books.

After the intellectual stimulation of much of this book, it is an anticlimax to read through the last 100 pages. We are given chapters on Inorganic and Organic Chemistry, most of which would be expected knowledge in a junior trainee, and should in any case be read before the earlier chapters. There is a useful chapter on Laboratory Miscellanea, and finally a section on Quality Control, much of which consists of charts to record, for example, temperature variations in paraffin wax baths.

This book is well presented with colour plates showing 48 stain examples and numerous drawings. I found large sections interesting reading and it has a useful place in the laboratory.

B.G.J.

Gynaecological Cytology: A Textbook and Atlas (1980). M. E. Boon and M. L. Tabbers-Boumeester. Published by the Macmillan Press Ltd, London and obtained from the Macmillan Company of New Zealand Ltd, Auckland. Three hundred and twenty-nine pages (192 pages text and 137 pages Atlas), illustrated, soft backed. Price: NZ$53.20.

Text: The first half of the 16 chapter text considers the benign aspects of gynaecological cytology, from cell biology to the anatomy, development and physiology of the female genital tract in the first four chapters. It continues up to chapter 8 discussing basic cytogenetics, hormonal effects and evaluations, and benign changes due to inflammation and infection.

The second half (chapters 8 to 16) deals with tumours of the female genital tract complete. Much thought is obviously offered to make the book flow and the inclusion of development and physiology helps the reader to understand the pattern of the female genital tract from its embryonic stage to changes at atrophy.

Each chapter is divided into sections and at all points the cell is viewed by its histology and electron microscopy, as well as cytology. Statistics confirm statements wherever possible in an easy to understand manner.

Inflammation and infections are dealt with thoroughly, although the virus section is perhaps too brief considering recent articles concerning their possible role with cervical cancer. However, this chapter is well concluded by proliferation and regeneration which completes the benign cytology.

There is little to be said concerning the chapters for the abnormalities of the female genital tract as these are very complete and introduced by a chapter on morphogenesis and morphology of tumour cells—a very valuable addition. Also good emphasis is made relating colposcopy to the diagnosis of cervical cancer. A chapter on technical methods is satisfactory and the text is ended with a short, but interesting chapter showing the authors' interpretation and methods of reporting cytology. References are clear and plentiful and listed at the end of each chapter.

The noticable failings of the text are the illustrations, which are all drawings and often do not provide the clarity of photography and in addition there is no cross reference to the Atlas. Also there is very little mention of the "C.I.N." (cervical intraepithelial neoplasia), concept which is popular with some cytologists.

Atlas: This section follows the same pattern as the text and throughout the photography is crisp, very representative and at sensible magnifications. Each cytological picture is complemented with histology, electron microscopy (T.E.M. and S.E.M.) and colposcopy, all with precise description. Fortunately, reference is made to the text. All are black and white and although colour would help the interpretation of the differential Papanicolaou stain, one must remember the reasonable price of the book.

To conclude, the book investigates all aspects of gynaecological cytology and includes the necessary basics which are essential to the understanding of the subject. The authors' modern approach is comprehensive, incorporating such subjects as colposcopy, electron microscopy and histology which help to understand and indeed, complements the often varied cytological picture.

"Gynaecological Cytology" is modestly priced and an extremely precise book for both tuition and laboratory reference; to suit the novice to the experienced and offers much knowledge needed in making an accurate diagnosis.

H. J. Neal

Abstracts

Shirley Gainsford, Maree Johnston, N. J. Langford, L. M. Milligan, Brian C. Thackeray, Errol Crutch, Harold Neal.

Haematology

A Sex Difference in the Bleeding Time. Bain, Barbara and Forster, T. (1980), *Thrombos. Haemostas., (Stuttgart)* **43,** 131.

The bleeding time by a modified Ivy technique was estimated on 128 normal volunteers. The bleeding time was found to be significantly longer in women than men. This was not attributable to a difference in platelet count nor to any detectable difference in platelet aggregability. It is likely that a difference in blood vessels or supporting tissues is responsible and may be related to the observation commonly made that women are far more likely than men to complain of excessive bruising.

Unreliability of Radiodilution Assays as Screening Tests for Cobalamin (Vitamin B12) Deficiency. Cohen, K. L. and Donaldson, R. M. (1980), *JAMA* **244,** 1942.

Recent studies have demonstrated problems with the radiodilution assay method for measuring serum Vitamin B12 levels. The authors have found the standard commercial radiodilution kit assays to be totally ineffective in screening for cobalamin deficiency. However they have found that some of the newer modified kits tend to give more equivocal results.

The Clinical Significance of the Antinuclear Antibody Test as a Screening Procedure for DNA Antibodies in SLE. El-Globarey, A. E., Sloane, D. J. P. and Whaley, K. (1980), *Scott Med J.* **25,** 293.

The authors consider the DNA binding capacity test an important tool in the diagnosis of SLE. However they did not think the level of the DNA binding capacity to be of any diagnostic value. They also state that the ANA test appears to be a valuable screening procedure.

A New Neutrophil Candida Killing Test: Chromium—51 Release from Candida guilliermondii. Martin, S., Ghoneim, A. T. M. and Child, J. A. (1980), *J. Clin. Pathol.* **33,** 757.

Neutrophil candicidal function was measured by a chromium—51 release technique. The effect of varying the neutrophil:candida ratio, the time course of ^{51}Cr release, the effect of different serum concentrations and the reproducibility of the technique were investigated and showed the technique to be a reliable and reproducible assay of candida killing.

Automated Platelet Counters. Mayer, K. *et al.* (1980), *Am. J. Clin. Pathol.* **74,** 135.

The authors have carried out an extensive evaluation of the following platelet counters: Coulter Model S plus, Ortho ELT-8, Baker MK-4/HC, and the Clay-Adams Ultra-Flo 100.

Heparin and the Activated Partial Thromboplastin Time: A Difference Between the In-vitro and In-vivo Effects and Implications for the Therapeutic Range. Bain, Barbara, Forster, T. and Sleigh, Barbara (1980), *Am. J. Clin. Pathol.* **74,** 669.

In choosing APTT reagent for heparin control, both threshold sensitivity and reactivity are important. The former determines whether there is good separation of adequately heparinized patients from those who are inadequately heparinised; the latter determines if adequately and excessively heparinised patients are readily separated. The authors compare seven APTT reagents.

ELISA Assay for Measurement of Human Haemoglobin A and Haemoglobin F. Makler, M. T. and Pesce, A. J. (1980), *Am. J. Clin. Pathol.* **74,** 673.

An ELISA assay for the measurement of nanogram quantities of haemoglobin A and haemoglobin F is described. This assay appears to be more sensitive than the immunoradiometric assay.

— E.R.C.

Prenatal Diagnosis of Haemophilia B by an Immunoradiometric Assay of Factor IX. Holmberg, L. *et al.* (1980), *Blood* **56,** 397.

An immunoradiometric assay of factor IX was developed based on homologous antibodies that arose in a haemophilic patient. Using this assay the authors diagnosed haemophilia B prenatally in one fetus at risk. After termination of the pregnancy, analysis of blood from the abortus confirmed the diagnosis of severe haemophilia B. Determination of factor IX coagulant activity in fetoscopic samples is unreliable because of possible contamination with thromboplastic material.

Standardisation of Factor VIII:C and Factor VIII R:Ag. Barrowcliffe, T. W. and Kirkwood, T. B. L. (1980), *Br. J. Haemat.* **46,** 471 and 483.

Two papers are presented from the National Institute for Biological Standards and Control, London. The authors discuss the techniques and controls necessary to produce standard Factor VIII:C and Factor VIII R:Ag preparations.

The Spleen—Hypersplenism. Richmond, J. (1980), *British Journal of Hospital Medicine* **24** 405.

The author defines hypersplenism and then discusses normal function, mechanisms of hypersplenism and indications for splenectomy.

The Normal Range of Osmotic Fragility of Red Blood Cells. Godal, H. C., Elde, A. T., Nyborg, N. and Brosstad, F. (1980). *Scand J Haematol* **25,** 107.

The authors carried out osmotic fragilities on 50 healthy subjects and found that nearly 20 percent fell outside Dacie's normal range. They suggest that the normal range should be wider than generally accepted.

Legionnaires' Disease in Leukaemic Reticuloendotheliosis. Berlin, G., Fryden, A., Maller, R., Malm, C. and Vikrot, O. (1980), *Scand. J. Haematol.* **25,** 171.

In the authors' hospital in Sweden 15 cases of leukaemic reticuloendotheliosis and four cases of Legionnaires' disease have been diagnosed. Three patients had both diseases. The authors state that it is probable that patients with leukaemic reticuloendotheliosis have an increased susceptibility to Legionnaires' disease.

— E.R.C.

Histology

Long Term Storage of Fresh Tissue at −20°C Embedded for Cryotomy. Miller, E. P. and Hogg, R. M. (1980), *Med. Lab. Sci.* **37,** 93.

A method is suggested which overcomes the difficulties encountered in the storage of unfixed tissues at sub-zero temperatures without desiccation and associated cutting and ice crystal artifact problems. Tissue blocks have been embedded in Ames OCT Compound Embedding Medium, frozen in liquid nitrogen, and stored at −20°C for up to 12 months with little distortion or loss of histochemical properties.

A Method of Preparing Paraffin Sections of Skeletal Muscle after Frozen Sectioning. Webb, J. N. and Green, J. J. (1980), *Med. Lab. Sci.* **37,** 91.

A method of preparing paraffin sections from blocks of frozen skeletal muscle tissue from which cryostat sections for routine histochemical studies have been taken. The authors claim that prior snap freezing produces superior results to immediate fixation.

Cost Saving with the Sorvall Resin Embedding System. Janes, R. B. (1980), *Med. Lab. Sci.* **37,** 179.

The author has overcome the problems of cost and space associated with filing specimens attached to their blockholders for future orientation and sectioning by substituting plain metal studs.

Technical Aspects of Lymphoma Immunohistology. David, Y., Biberfeld, P. (1980), *J. Histochem, Cytochem.* **8,** 731.

Immunohistologic Studies of Lymphoma: Past, Present and Future. Taylor, C. R. (1980), *J. Histochem, Cytochem.* **8,** 777.

Two papers from a special issue of this journal

devoted to the 1980 Histochemical Symposia conducted at the 31st annual meeting of the Histochemical Society held in New Orleans, 11-15 April 1980.

These papers provide a technical and pathological update in immunohistology as well as a tempting insight into future developments. There is also a wealth of information for the novice.

— B.C.T.

Management

Laboratory Computing—An Evaluation, with Guidelines for Potential Users. Garner, S. E. and King, G. A. (1980), *Med. Lab. Sci.* **37,** 215.

The planning and introduction of a computer system in a pathology laboratory is described, together with recommendations for potential users. The system is evaluated in respect of cost, staff considerations, expected and actual performance.

Data Capture by the Use of Bar-Coding in a Blood Transfusion Centre. Ibbotson, R. N. and Jackson, R. E. (1980), *Med. Lab. Sci.* **37,** 237.

A system of data capture using bar-coding and light sensitive pens attached to visual display units and a computer is described. The system being developed will record the receipt of blood donations, results of laboratory testing and the production and issue of blood components.

Microprocessors and Automation in the Medical Laboratory. Pritty, D. W. (1980), *Med. Lab. Sci.* **37,** 197.

In order to understand the impact of microprocessors on the medical laboratory, one needs to look closely at the characteristics of the microprocessor systems and to evaluate the relevant uses for such systems.

— L.M.M.

Immunohaematology

Human Blood Components. 1. Preparation and Characteristics of Leucocyte Concentrates from Single Units of Human Blood. Waldman, A. A. and Shander, C. (1979) *Trans.* **20,** 4.

A detailed study of the distribution of white blood cells remaining on the ACD and CPD anticoagulated packed cells after the removal of the platelet rich plasma, was carried out. The effective employment of the leucocyte preparation procedures, requires methods to allow harvesting without compromising the preparation of other blood products is discussed.

Blood for Use in Exchange Transfusion in the Newborn. Barnard, D. R., Chapman, R. G., Simmons, M. A. and Hathaway, W. E. (1980) *Trans.* **20,** 4.

Biochemical and coagulation parameters were studied in fresh whole CPD blood, whole blood stored for four days and blood reconstituted from four-day-old packed cells with fresh frozen plasma. Preliminary observations are reported of exchange transfusions on infants receiving one of the mentioned variant products.

An Additional Example of Autoanti-M. Vale, D. R. and Harris, I. M. (1979) *Trans.* **20,** 4.

Another example of a potent autoanti-M is described in a non-gravida, non-transfused Caucasian woman. The anti-M was still present after a period of 12 months without any apparent cause.

In Vitro Evaluation of Platelets Stored in CPD-Adenine Formulations. Bolin, R. B., Cheney, B. A., Simpliciano, O. A. and Peck, C. C. (1979), *Trans.* **20,** 4.

In order to evaluate the effect of adenine and added glucose on stored platelets, CPDA-2 and CPDA-3 were used as storage medium. The pH, morphology score platelet size and distribution parameters were analysed. The results suggest that adenine and added glucose in these anticoagulant solutions do not adversely affect the in vitro reaction of the platelets.

— L.M.M.

Cytology

A Review of the Past and Future of the Society and of Cytology. Alan, B. P. (Guest Editor) (1977), *Acta Cytologica* **21,** 605.

A collection of various articles submitted by different authors to commemmorate the 25th Anniversary of the American Society of Cytology.

The history and future of cytology is reviewed and discussed, including the education and the role of both the pathologist and cytotechnologist in the subject.

A Comparative Cytologic Study of 100 Urine Specimens Processed by the Slide Centrifuge and Membrane Filter Technique. Marway, Shachi, Devlin, Donna and Dekker, A. (1978), *Acta Cytologica.* **22,** 431.

One hundred consecutive urines were processed by both the Slide Centrifuge and Membrane filter Technique. The comparison showed that the cell recovery and quality was more superior when using the Membrane Filter Techniques. However, no false positive cases were recorded with either method showing that both methods gave a high degree of accuracy.

Cytological Investigations in Chlamydia Infection. Gupta, P. K., Less, E. F., Erozen, Y. S., Frost, J. K., Geddes, Susan T. and Donovan, Patricia A. (1979), *Acta cytologica.* **23,** 315.

This paper describes the presentation and cytomorphology of chlamydia infection in the cervical smear and confirms diagnosis by immunofluorescence, electron microscopy, histochemistry and tissue culture. The clinical implications of this common genital infection are discussed.

Assessment of Three Sampling Techniques to Detect Endometrial Cancer and its Precursors. Bibbo, M., Reale, F. R., Reale, Joyce C., Azizi, F., Bartels, P. H., Wied, G. L., Hajj, S. N. and Herbst, A. L. (1979), *Acta Cytologica.* **23,** 353.

A comparison of three techniques for the collection of material from the endometrium was made. These were Endometrial Aspiration (Vakutage) Endocervical Aspiration and vaginal, ectocervical and endocervical smears (VCE), all being outpatient procedures. It was shown that in all cases, benign to carcinoma, that the direct endometrial aspiration gave the highest yield of detection, being 100% with the carcinomas. The study also showed that by combining the endometrial aspiration with either the VCE smears or the endocervical aspirations, no significant increase in the detection rates were seen.

Colposcopy: A Medical Screening Method. Herbeck, G. (1980), *Zeiss Information* **24,** 39.

This short article points out the value of colposcopy in the detection of cervical cancer especially for early abnormal changes. It also emphasises that cytology and colposcopy complement each other and when used together, increases the detection rate of early treatable cervical cancer. The article is supplemented with colour colpophotographs.

— H.J.N.

Microbiology, Virology and Public Health

Nontuberculous Mycobacteria as Unsuspected Agents of Dermatological Infections: Diagnosis Through Microbiological Parameters. Damsker, B. and Bottone, E. J. (1980), *J. clin. Microbiol.* **11,** 569.

Over a 10 year period, seven cases of skin lesions caused by nontuberculous mycobacteria occurred. Details of the patients and the isolates are given. Initially the mycobacterial aetiology was not suspected and our attention is drawn to these organisms as causative agents of skin infection.

Identification of Nocardiae and Streptomycetes of Medical Importance. Mishra, S. K., Gordon, Ruth E. and Barnett, Dorothy A. (1980), *J. clin. Microbiol.* **11,** 728.

The authors identified 658 clinical isolates of aerobic gram positive organisms with branching filaments. From their results, two tables of properties and a key for tentative identification of these organisms has been devised. These will help the routine laboratory to identify these organisms.

2-Mercaptoethanol Brucella Agglutination Test : Usefulness for Predicting Recovery from Brucellosis. Buchanan, T. M. and Faber, L. C. (1980), *J. clin. Microbiol.* **11,** 691.

The standard brucella agglutination test and 2-mercaptoethanol (2ME) brucella agglutination test were performed on 92 patients over 18 months on 15-25 sera from each patient. The standard brucella agglutination test remained positive for 18 months in half the patients despite antibiotic treatment, whereas the 2ME test was positive in

only four patients. A negative 2ME test therefore meant a favourable response to treatment and strong evidence against chronic brucellosis.

Rapid Identification of Staphylococcus aureus by Using Lysostaphin Sensitivity. Severance, P. J., Kauffman, C. A. and Sheagren, J. N. (1980), *J. clin. Microbiol.* **11,** 724.

A gram stain of a broth culture of staphylococcus pre- and post-exposure to lysostaphin was used as a screening test for differentiating *Staph aureus* from other staphylococci and micrococci. A reduction of 90% or more in the number of organisms seen in comparing the pre- and post-treatment gram stain was a positive result. This test could be used to detect Staph aureus in blood cultures and other body fluids.

Evaluation of an Enzyme Immunoassay for Serum Gentamicin. O'Leary, T. D., Ratcliff, R. M. and Geary, T. D. (1980), *Antimicrob. Agents Chemother.* **17,** 776.

This is an evaluation of the reproducibility and specificity of the Enzyme Multiplied Immunoassay Technique (EMIT) for serum gentamicin levels. The method compared favourably with microbiological assay and gave quicker results.

Evaluation of the Phadebact Gonococcus Test for Confirmation of Neisseria gonorrhoeae. Anand, C. M. and Kadis, Eileen M. (1980), *J. clin. Microbiol.* **12,** 15.

Colonies on Thayer Martin media and media with and without serum were tested by the direct method of Phadebact Gonococcus Test. Most strains were difficult to emulsify and reactions hard to interpret. Using the alternative proceedure of boiling the strain for five minutes first, a suspension free of clumps was obtained and 427 out of 432 stains of N. gonorrhoeae were identified. False positive results were obtained with N. lactamica and therefore it is recommended that the ONPG test is done in conjunction with the Phadebact Gonococcus Test.

Rapid Determination of Minimum Inhibitory Concentrations of Antimicrobial Agents by the Autobac Method: A Collaborative Study. Schoenknecht, F. D., Washington II, J. A., Gavan, T. L. and Thornberry, C. (1980), *Antimicrob. Agents Chemother.* **17,** 824.

The authors report on the results obtained by four laboratories who evaluated the Autobac minimum inhibitory concentration system. One thousand, two hundred and sixty clinical isolates were tested against 10 antibiotics by the Autobac system and a modified International Collaborative Study broth dilution technique. There was an agreement of 95% between the two methods. The main disagreement occurred with Penicillin G and staphylococci.

Biochemical Properties of CO_2 Dependent Streptococci. Pulliam, L., Perschen, R. K. and Hadley, W. K. (1980), *J. clin. Microbiol.* **12,** 27.

This is a study of 153 clinical isolates and 10 reference strains of CO_2 dependent streptococci. Most strains were non-reactive serologically but biochemical tests using a basal medium of thioglycollate with 0.1% Tween 80 to give luxuriant growth proved a good means of differentiating and identifying this group of streptococci.

New Methods for the Isolation of Legionella pneumophila. Greaves, P. W. (1980), *J. clin. Pathol.* **33,** 581.

An enriched blood agar, selective blood agar and enrichment broth are described. This media were all more sensitive than FG, CYE, and Mueller Hinton agar containing haemoglobin and isovitalex in the isolation of Legionella pneumophila. L. pneumophila was recovered from 6 out of 10 patients with clinical evidence of Legionnaires using combinations of these media.

An Evaluation of the ToxHA Test for the Detection of Antibodies to Toxoplasma gondii in Human Serum. Balfour, A. H., Bridges, J. B. and Harford, J. P. (1980), *J. clin. Pathol.* **33,** 644.

A commercially available kit test (Tox HA test-Wellcome Reagents Ltd) was compared with the dye test in the detection of toxoplasma antibodies.

One thousand, nine hundred and eighty-five sera were tested. Thirteen recent cases of infection were not detected and there were considerable sera with a negative dye test and positive HA test. The authors suggest that some of the specificity may be directed against non-toxoplasma constituents of the antigen used in the red cell sensitisation. The test is simple to perform and may be mechanised.

Isolation and Identification of Haemophilus ducreyi in a Clinical Study. Sottnek, F. O., Biddle, J. W., Kraus, S. J., Weaver, R. E. and Stewart, J. A. (1980), *J. clin. Microbiol.* **12,** 170.

Chocalate agar enriched with Isovitalex, chocolate agar plus vancomycin, rabbit blood agar plus vancomycin, fetal bovine serum agar and fetal bovine serum agar plus vancomycin were all compared as primary isolation media for the isolation of *Haemophilus ducreyi* from genital lesions. As well sera from five animal species and human sera were tested for their ability to support the growth of H. ducreyi. All the culture media supported good growth of *H. ducreyi* particularly with the addition of vancomycin. Fetal bovine serum was the only serum to support growth of all 17 strains tested. Biochemical and physiological studies were done using conventional media plus fetal bovine sera and their results plus those of two reference strains of H. haemoglobinophilus are given.

Rapid Tube CAMP Test for Identification of Streptococcus agalactiae (Lancefield Group B). Phillips, Edna A., Tapsall, J. W. and Smith, D. D. (1980), *J. clin. Microbiol.* **12,** 135.

A rapid CAMP test to identify Streptococcus agalactiae in four hours is described. The supernatants from four hour broth cultures of streptococci were neutralised then added to sheep red blood cells and staphylococci beta lysin. A positive result was shown by 50% haemolysis in 10 minutes.

Enzyme-Linked Immunosorbent Assay for Quantitation of Toxoplasma Antibodies in Human Sera. Am Van Loon and Van Der Veen, J. (1980), *J. clin. Pathol.* **33,** 635.

Antigen from Toxoplasma gondii cultivated in human cell cultures was used to develop an ELISA to detect toxoplasma antibodies. A standard curve was constructed so that the antibody titre of patients' sera could be obtained using one serum dilution of 1:800. The reproducibility of the test was good although with some microtitre plates the "edge phenomena" was seen, possibly caused by the manufacturing process of the plates. The ELISA corresponded well with the immunofluorescence test and complement fixation test.

ELISA for Toxoplasma Antibody Detection: A Comparison with Other Serodiagnostic Tests. Balsari, A., Poli, G., Molina, V., Dovis, M., Petruzelli, E., Boniolo, A. and Rolleri, E. (1980), *J. clin. Pathol.* **33,** 640.

The authors developed an ELISA method for measuring toxoplasma IgG antibodies then examined its sensitivity and specificity by comparing it with the dye test, indirect haemagglutination (HA), indirect immunofluorescence (IF) and crossover linked immunoassay. The specificity was comparable with all these tests and sensitivity with the HA and IF tests.

Detection of Chlamydia trachomatis in Rapidly Produced McCoy Cell Monolayers. Evans, R. T. and Taylor-Robinson, D. (1980), *J. clin. Pathol.* **33,** 591.

After treating coverslips with glutaraldehyde-activated-y-aminopropyl-triethoxysilane, confluent monolayers of cells could be produced in less than one hour instead of the usual 24 hours with untreated coverslips. Monolayers of cells on treated and untreated coverslips were inoculated with 13 *Chlamydia trachomatis* serotypes and clinical specimens. When there were large numbers of inclusions, more were always seen in the untreated coverslips but when there were few inclusions, more were seen in cells on the treated coverslips than on untreated coverslips. The treated coverslips still formed monolayers of cells after storage at room temperature for 15 months.

— S.G.

Directions for Contributors

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

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

Illustrations

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

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

Nomenclature and Units

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

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

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

N.B.:

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

2. No space should be left between the symbol for a prefix and the unit. A space is left between the symbols in derived units.

e.g., ms = millisecond
m s = metre x second

Where ambiguity could arise words should be written in full.

3. **Numbers.** The decimal is indicated by a full stop. Commas are not used to divide large numbers but a space is left after every third digit.

A zero should precede numbers less than unity. Units which give a number between 0.1 and 1000 should be chosen when possible.

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

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

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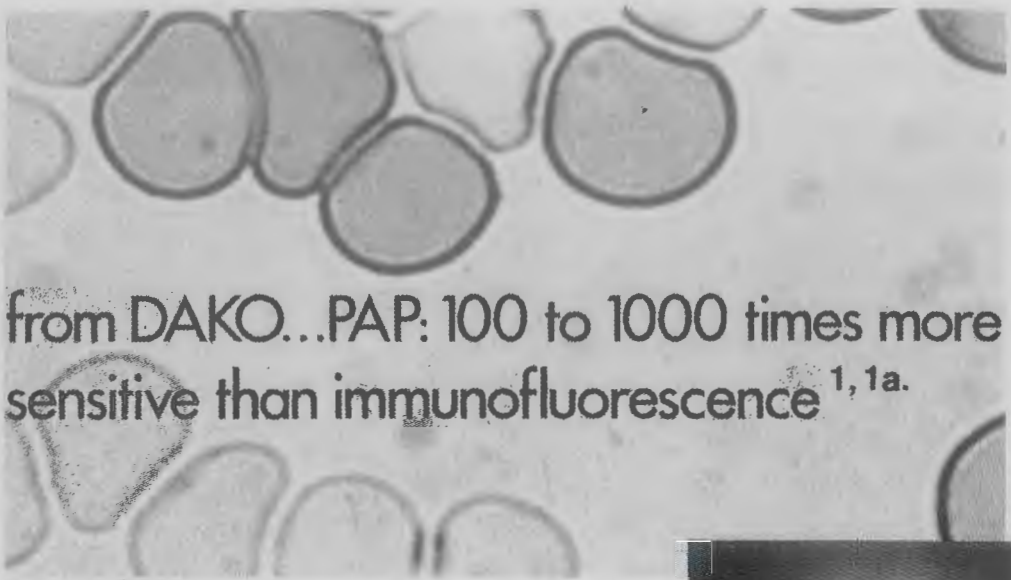

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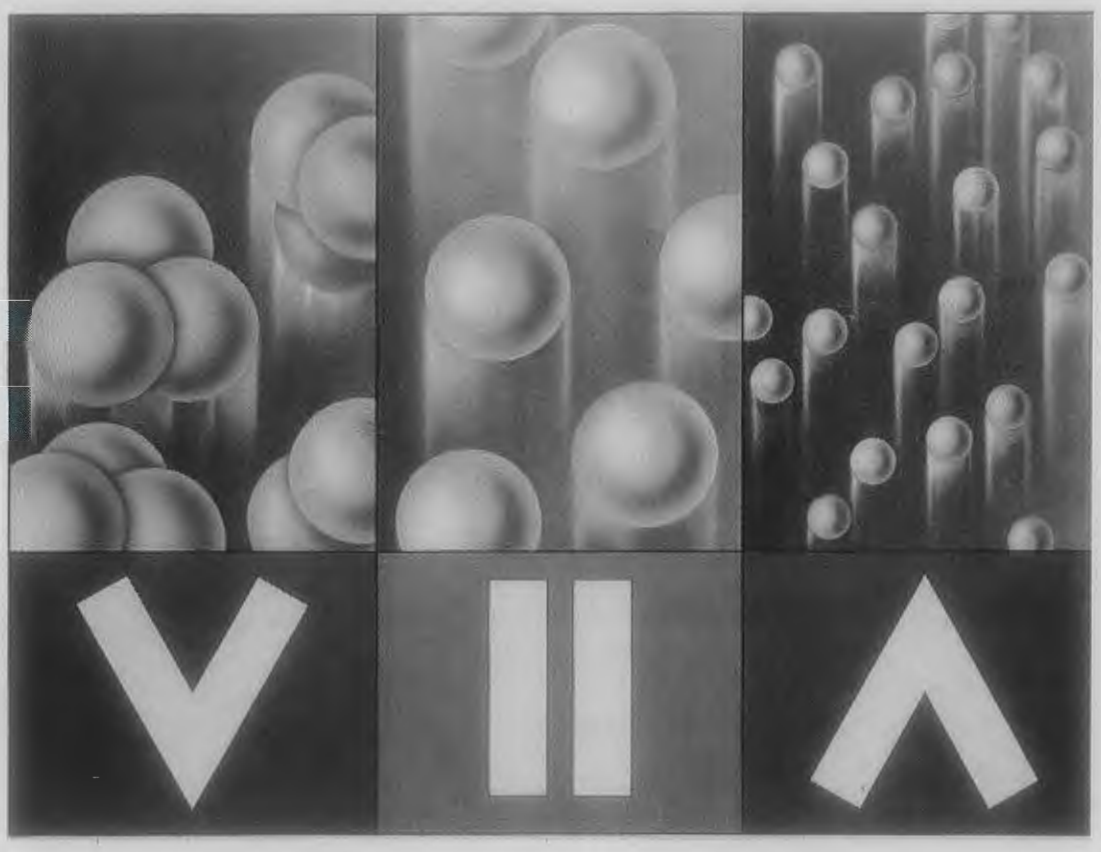

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