

JULY, 1976



THE NEW ZEALAND JOURNAL OF

medical laboratory technology

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

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VOLUME 30, NO. 2

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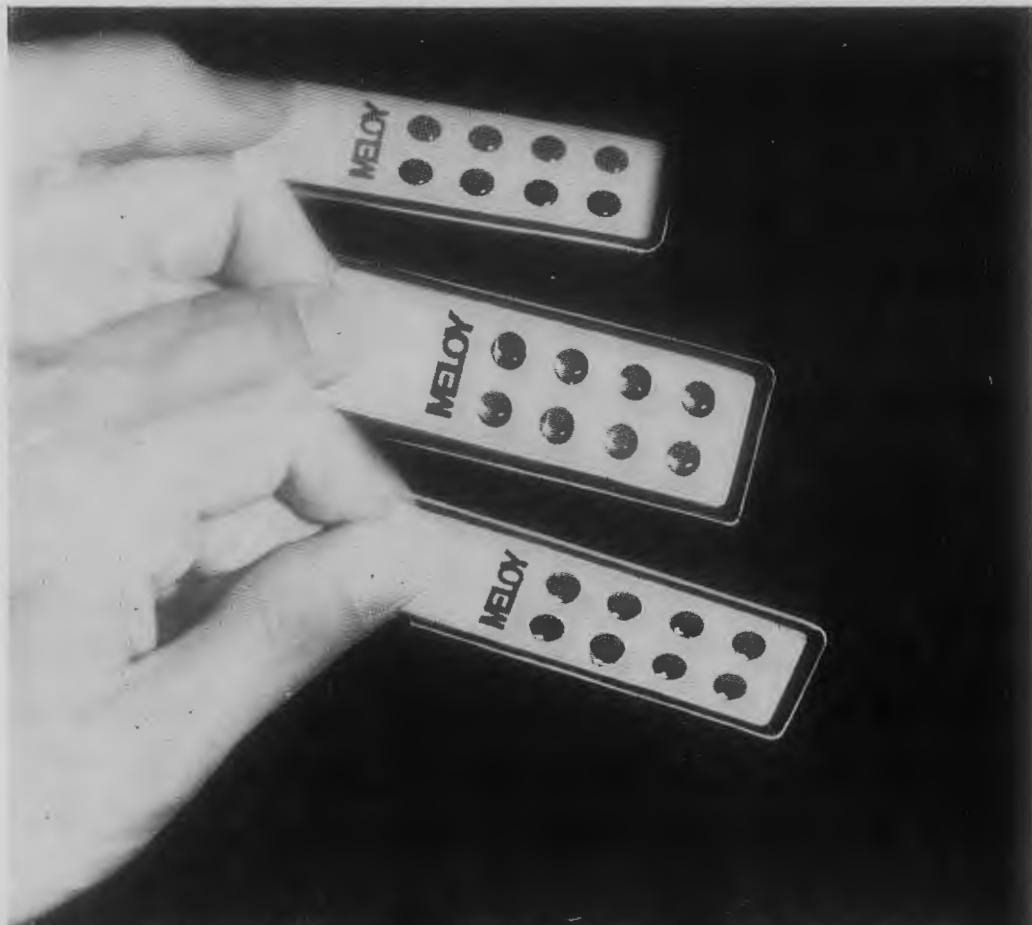
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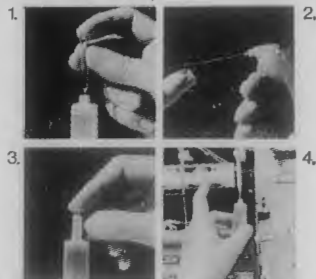
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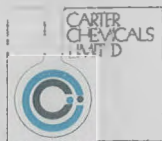
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The New Zealand Journal of Medical Laboratory Technology

Volume 30, No. 2

July, 1976

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Laboratory Technology (Inc.)



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

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

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Report of a Severe Case of Methaemoglobinaemia Complicated by Sonnei Dysentery

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Received for publication, October 1975

Introduction

Nitrogen is widespread in nature. Nitrate is an inorganic anion formed by oxidising elemental nitrogen. It is an essential nutrient of plants and it is utilised by plants to form plant protein. Whenever one of these biologic systems is upset, nitrate may then accumulate in the soil, water, or plants. This can lead to nitrate poisoning which in humans, chiefly infants, takes the form of brownish-blue discoloration, first noticed around the lips, spreading to the fingers and toes and over the face and eventually covers the whole body. Other signs include drowsiness, increased respiration and death. The clinical signs of acute nitrate toxicity are attributed to anoxia, and appear when the methaemoglobin levels reach 30 percent - 40 percent. Death occurs when methaemoglobin levels reach 70 percent - 90 percent. The nitrate ion is reduced to nitrite, which then oxidises the iron of the haemoglobin molecule from the ferrous to the ferric form. The resulting methaemoglobin is incapable of reversibly binding molecular oxygen. Some haem ion is always being spontaneously oxidised to the ferric state. As long as this proceeds at a normal rate, the enzyme system is able to maintain the bulk of the iron in the ferrous form, with the result that normally less than 1.5 percent of the red cell Hb is in the methaemoglobin form.

Case History

A case is presented below in which methaemoglobinaemia was complicated by *Shigella sonnei* dysentery.

A thirteen-month-old Maori child was admitted to Kaitaia Hospital on 26 December 1974 suffering from diarrhoea and vomiting. He was isolated and requests made for full blood count, urea and electrolytes. The only abnormalities were:

Hb 9.2g/100ml MCHC 25%

PCV 37% WBC 21,300 = 48% Neutroph Seg.

A rectal swab was taken from which *Shigella sonnei* was isolated and the ward notified. The

child was progressing well, when suddenly on 29 December, he went into shock and respiratory collapse and died within thirty minutes despite cardiac massage and oxygen administration. Later that same afternoon a cousin of the dead child, who also had been admitted with diarrhoea, collapsed and went into respiratory failure. Swift action on the part of the ward staff and the fact that the child was older helped save him. He remained on the critically ill list for two to three days. The laboratory was called on this occasion and blood obtained by femoral stab by the house surgeon. It was noted that the blood appeared like chocolate agar and the comment was made by the house surgeon at the time "Perhaps it is methaemoglobin". This child was also anaemic Hb 9.4g/100ml, PCV 34% MCHC 28% with a white blood count 10,500. Urea and electrolytes were normal.

Due to the manner in which the first child had died it was decided that a post-mortem examination was to be performed. The body was taken through to Auckland where Dr Cairns examined it and reported to the medical superintendent that the child had died of severe methaemoglobinaemia complicated by Sonnei dysentery. The sequestrene blood from the second child had also been sent to Professor Cairns. The two methaemoglobin levels were:

Deceased child	50%
Cousin of deceased	18%
(Blood twenty-four hours old when examined)	

An investigation was begun immediately to trace the source of the nitrate and/or nitrite contamination which was suspected of causing the methaemoglobin. It was found that the dead child was a very heavy drinker of water so that the community water supply, a raw supply from the Herekino State Forest, which is reticulated to six homes, was at first suspect.

Blood samples were obtained from seventy-five persons who had been "at risk," i.e., had been in or into the household from which the dead child came. They were all tested

for haemoglobin, methaemoglobin and percentage methaemoglobin calculated. From the seventy-five persons a total of ten produced a methaemoglobin level greater than 5 percent. Most estimations were performed within 2h of taking the blood. Two members of the laboratory staff used as controls had no measurable methaemoglobin.

This proved to be an exercise involving laboratory, hospital and Health Department staff. A student doctor from Singapore was co-opted to go out with the district health nurses to bleed all the patients. The contacts in hospital were bled by the laboratory staff. These people were hospitalised on the advice from the medical superintendent as a precautionary measure because they had *Shigella sonnei* dysentery or symptoms of *Shigella sonnei* dysentery. At the time of the child's death it was decided that rectal swabs should be taken from all contacts of the child and all visitors to the household concerned. At this time of the year this amounted to in excess of two hundred swabs and necessitated help in making of media and pouring of plates.

While this outbreak of *sonnei* was being investigated at Manukau about 18km from Kaitaia, an outbreak of *Salmonella typhimurium* dysentery was found on the east coast about 40km from Kaitaia. This had apparently started there on Christmas day after the guests had been served left-over vegetables from Christmas dinner. The first person to go down with it was a member of the staff, followed by other staff members and guests.

To this date no source has been found of any contaminating nitrate, nitrite or for the *Shigella sonnei*. Samples of water taken were examined bacteriologically at Kaitaia Hospital and chemically by the DSIR, Auckland. The DSIR reported that there was no concern with regard to nitrite or nitrate levels from either of the two homes involved. The bacteriological reports were:

- (1) Home A Manukau communal raw supply from Herekino State Forest native bush. No coliform organisms.
- (2) Home B Wainui Junction
 - (a) Roof catchment to storage tank supply, 17 probable coliform bacilli/100ml. 3 typical *E. coli*.

- (b) Stagnant water in cattle drinking trough adjacent to the house, 35 probable coliform bacilli/100ml not typical *E. coli*.

The local health inspector in his report tends to suspect that there has been latent *sonnei* infection in home (A) since November 1974 when one member of the family complained of a stomach upset. She was notified as food poisoning, unspecified at that time. Alternatively the source of infection possibly arose from a member of another family arriving north from Auckland for Christmas.

Other possible causes of high nitrate or nitrite by ingestion have included:

1. Alternative, but polluted, water supplies, none apparent.
2. Vegetables high in nitrate/nitrite.
3. Preservatives.
4. Mis-use of medications, in tablet or pill form, including paracetamol or phenacetin.

All of these causes were investigated but turned out negative.

The possibility of a hereditary haemoglobinopathy or red cell enzyme defect was considered and specimens from the families involved were examined in Wellington and Auckland for methaemoglobin reductase deficiency. Equivocal results were obtained. Specimens from one of the family were examined in Christchurch for methaemoglobin reductase and haemoglobin stability and were screened for increased oxidative susceptibility. All of these tests were normal. Dr R. W. Carrell, Associate Professor, Clinical Biochemistry, Christchurch Hospital, suggested adding dye substances and chlorate to the list of possible causes of a secondary methaemoglobinaemia.

Although no conclusion could be arrived at, this unusual occurrence seemed worth recording.

Acknowledgments

I wish to thank Anne Southern, Mr R. Anderson and Dr R. W. Carrell for the laboratory investigations; Mr L. Jackson, County Health Inspector; Miss J. Bell and Mrs M. Mahalovich, Public Health Nurses; the laboratory staff and Dr D. Tree, Medical Superintendent, Kaitaia Hospital. All of these people were involved in this investigation.

Yeast-like Fungi Other Than *Candida albicans* in Clinical Specimens

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Received for publication, January 1976

Introduction

The pathogenicity of *Candida albicans* has been established¹ and the role of this organism in clinical conditions is well known.

Further studies⁷ in later years showed that other members of the genus *Candida* could be implicated in clinical conditions similar to those brought about by *Candida albicans*. Possibly through a lack of simple laboratory methods, little has been published on the incidence and importance of yeast-like fungi other than the genus *Candida*. As *Candida albicans* and subsequently other members of the genus *Candida* have been shown to have opportunistic infective properties it seems logical to expect that yeast-like fungi in general should have similar pathogenicity though perhaps of a lower order because of the lower incidence of these organisms in the human habitat.

The increased use of broad spectrum antibiotics has been shown^{9, 4} to be a factor predisposing the patient to secondary infection with *Candida*, particularly of the oral and alimentary tract.

Diabetes⁶ and steroid therapy¹¹ have also been shown to increase the chance of a person being infected with *Candida*. The current increase in the use of immunosuppressive drugs increases the chance of infections from many hitherto unimportant organisms. Any yeast-like organisms present could take advantage of the lowered body resistance under these conditions and set up disease processes that may, in the extreme case, be life threatening if widespread.

All yeast-like fungi must be considered worthy of investigation for these foregoing reasons. With the development of simpler laboratory techniques and schema in recent times^{2, 3, 5} more accurate identification of yeast-like fungi of medical importance has now come within the reach of clinical microbiology laboratories.

Materials and Methods

One thousand, two hundred and twenty

yeast-like fungi (Table I) isolated from clinical specimens were used. Most of these were isolated from vaginal swabs, the remainder being from swabs of other sites, skin and nail scrapings (Table II). All the organisms were isolated in Sabourauds dextrose agar with antibacterial antibiotic (Gentamicin 100 μ g/ml) but, without cycloheximide (Acti-dione — Upjohn), as many yeast-like fungi are sensitive to this antibiotic.

All yeast-like organisms were screened using the germ-tube test¹⁰ and those producing germ-tubes, in three hours at 37°C, were designated as *C. albicans*. The other yeast-like organisms were identified by the scheme of Dolan² with certain modifications (Table III). *Rhodotorula* species were identified by the presence of carotenoid pigmented (orange to coral red) yeast-like colonies and not investigated further.

Rice-Tween 80 agar (BBL) was used for microscopic colonial morphology instead of cornmeal-Tween 80 agar and was found to be satisfactory.

Sugar assimilations were carried out by adding 1.5ml of 10x strength yeast nitrogen base (Difco), previously sterilised by membrane filtration (Millipore GS), to 15ml of sterile molten 2 percent agar (Davis) which had been cooled to approximately 50°C, and poured into a petri dish. It was found convenient to make up 2 percent agar and dispense in 15ml amounts to bottles before autoclaving at 121°C for 15m so that one petri dish could be poured as required. The plate was inoculated by flooding the surface of the medium with a few ml of sterile distilled water made slightly turbid with a portion of the yeast-like colony from Sabourauds agar. After draining the excess fluid off the plate, the surface was dried for an hour before placing sugar impregnated discs (Difco) on the agar surface. Assimilation is indicated by heavier growth around the discs after incubation at 28°C for 24 to 48h. The pattern is read when the optimum growth is obtained. If the

Table I. Yeast-like fungi isolated.

Identity	Number of Strains	Percentage of total
<i>C. albicans</i>	1041	85
<i>Torulopsis</i> sp.	62	5.1
<i>Rhodotorula</i> sp.	51	4.2
<i>T. glabrata</i>	25	2.2
<i>C. krusei</i>	23	1.9
<i>C. parapsilosis</i>	7	0.7
<i>C. guilliermondii</i>	4	0.3
<i>C. species</i>	4	0.3
<i>Saccharomyces</i> sp.	2	0.2
<i>C. tropicalis</i>	1	0.1
TOTAL	1220	

Table II. The Frequency of Various Yeast-like Fungi isolated from Different Sites.

Organism	Vagina	Skin	Nail	Mouth and Throat	Ear	Sputum
<i>Torulopsis</i> sp.	25	29	1	5	-	2
<i>Rhodotorula</i> sp.	11	40	-	-	-	-
<i>T. glabrata</i>	23	-	-	1	-	1
<i>C. krusei</i>	10	5	4	-	4	-
<i>C. parapsilosis</i>	1	4	-	-	2	-
<i>C. guilliermondii</i>	1	2	1	-	-	-
<i>C. species</i>	2	3	-	-	-	-
<i>Saccharomyces</i> sp.	-	1	-	1	-	-
<i>C. tropicalis</i>	1	-	-	-	-	-

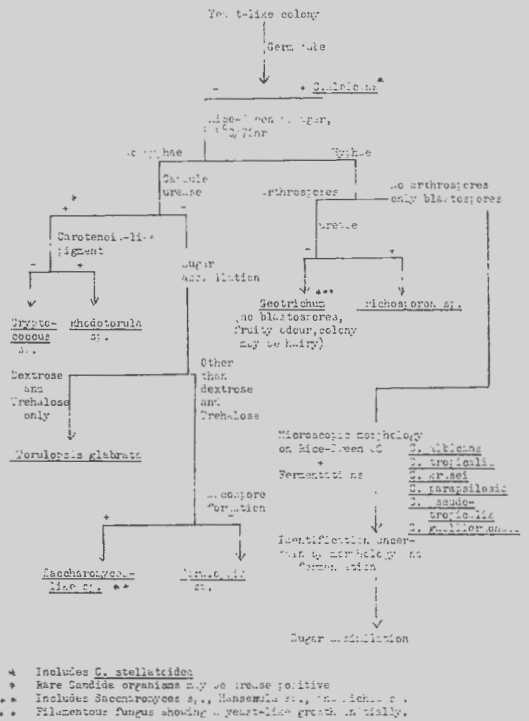
inoculum is either too heavy or too light the zones of growth around the discs may be difficult to interpret and one has to be prepared to repeat the assimilations under these circumstances.

Candida species were identified on the basis of microscopic morphology and sugar fermentations. If the results seemed equivocal sugar assimilations were also done.

For fermentation studies, 3 percent sugars were used in bromcresol purple broth base (Fort Richard Laboratories Ltd prepared media) and after inoculation with the organism, incubated at 28°C for 10 days. Fermentation patterns and other characteristics were compared with the differential characters of the yeast-like fungi as shown (Table IV).

Ascospores were stained by the method of McClung (1943)⁸, by making thin smears of the growth from acetate ascospore agar², heat fixing, flooding with 1 percent malachite green in 1 percent aqueous phenol and

Table III. Sequential chart for identification of Yeast-like fungi - after Bolan¹ mod.



* Includes *C. stellatoidea*
 † Rare *Candida* organism may be urease positive
 ‡ Includes *Saccharomyces* sp., *Hansenula* sp., etc.
 ††† Filamentous fungus showing a yeast-like growth initially.

heating over a small flame (with steaming but not boiling) for two minutes. The slide was then washed gently under the tap for one minute, then counterstained with dilute carbol-fuchsin (1 in 10 to 1 in 20 Ziehl-Neelsen carbol-fuchsin) for a few seconds. By this method the ascospores are stained green, and the walls of the asci and vegetative cells are red. The preparations are clear cut and easy to read.

In addition to determining the incidence of yeast-like fungi, other than *Candida albicans*, an attempt was made to correlate laboratory isolations with clinical conditions. To this end a questionnaire was sent to consultants to determine specifically whether there was:

- any sign or symptom suggesting a yeast infection;
- any condition predisposing to yeast infection, e.g., diabetes;
- any therapy predisposing the patient to yeast infection, e.g., antibiotics, hormones, etc.;
- any symptomatic improvement subsequent to antifungal treatment.

Table IV. Differential characters of medically important members of the genus *Candida*.

	Colonial Morphology on Sab. dex.	Microscopic Morphology on Rice-Tween 80	Rice-Tween 80							Comment
			Carbohydrate reactions (a)							
			Glucose	Maltose	Sucrose	Lactose	Galactose	Raffinose	Trehalose	
<i>C. albicans</i> *	high convex colony	chlamydo-spores	AG +	AG +	A +	-	+	-	+	
<i>C. guilliermondii</i>	flattish, shiny	hyphae may be difficult to demonstrate	AG +	- +	AG +	-	+	+	+	
<i>C. krusei</i>	flat, dry, fringed	poor elongated blasto-spores "crossed matchsticks"	AG +	- -	- -	-	-	-	-	Marked surface film in fermentations
<i>C. parapsilosis</i>	active, flattish colony "vanilla-like odour"	"giant cells, hyphae"	AG +	- +	A +	-	+	-	+	Cloudy supernatant in fermentations
<i>C. pseudotropicalis</i>	fringed	elongated cells - like logs in stream	AG +	- -	AG +	AG +	+	+	-	
<i>C. tropicalis</i>	raised, sometimes wrinkled, fringed	like seaweed	AG +	AG +	AG +	-	+	-	+	abundant gas and surface film

* *C. stellatoidea* may be differentiated from *C. albicans* on the basis of a sucrose assimilation test. *C. stellatoidea* in contrast to *C. albicans*, does not assimilate sucrose (a). Cf. the two rows of results given for each species, the upper row shows carbohydrate fermentation reactions and the lower row indicates assimilation reactions.

Key: AG = acid and gas production (fermentation). A = acid production (carbohydrate assimilated only).
+ = carbohydrate assimilated. - = carbohydrate not assimilated or negative fermentation reaction.

This investigation was limited in that it was applied only to the latter part of the series studied and only to those patients showing fungal elements on direct microscopy of the material submitted. Fungal infections diagnosable by direct microscopy were considered likely to be more significant clinically than those requiring cultural methods for diagnosis.

Results

The percentage of the different yeast-like fungi identified from 1,220 isolates is shown in Table I. As might be expected most (85 percent) of the yeast-like fungi isolated were *C. albicans*. The sample is too small to show any statistically significant propensity of any one species for a particular site except for *T. glabrata* in the vaginal area; and *Rhodotorula* sp. in the superficial squamous epithelium. The distribution is shown in Table II.

The results of the clinical evaluation are shown in Table V. Of 19 questionnaires sent to clinicians 15 were returned sufficiently complete for evaluation.

Discussion

As might be expected the difficulties experienced in establishing the clinical significance of *C. albicans* are also encountered in any attempted assessment of the clinical role of yeast-like fungi other than *C. albicans*. These results indicate that some isolations of yeast-like fungi other than *C. albicans* are clinically significant and that they can cause symptoms which remit following appropriate treatment, i.e., with antifungal agents.

From a technical point of view, it is not too difficult for many microbiology laboratories to identify yeast-like fungi accurately. By the method described, identification to at least the genus level and in many cases to the species, is possible. The germ-tube test is widely used to identify *C. albicans* and the common practice of naming yeast-like fungi not producing germ-tubes as "*Candida species*" may be quite wrong. The results in this survey have shown that these organisms often belong to quite another genus apart from *Candida*, e.g., *Torulopsis*. In smaller laboratories where accurate identification of

Table V.

Organism isolated	Site	Symptoms of Yeast Infection	Predisposing Condition	Prior Therapy	Remarks
<i>Torulopsis</i> sp.	throat	exudative tonsillitis not clearing with Ampicillin	-	Ampicillin	Exudate cleared spontaneously with remission of glandular fever which was subsequently diagnosed.
<i>Torulopsis</i> sp.	vagina	discharge - not typical of a yeast	-	-	Symptoms cleared with Nystatin.
<i>Torulopsis</i> sp.	vagina	discharge and irritation	I.U.D.	-	Nystatin prescribed - patient not returned.
<i>Torulopsis</i> sp.	vagina	pruritus vulvae and discharge	-	-	Symptoms cleared with Nystatin and Pimafucin.
<i>T. glabrata</i>	vagina	vaginal discharge	-	Ampicillin and Bactrim	Nystatin prescribed.
<i>T. glabrata</i>	cervix	vaginal discharge and irritation	-	On oral contraceptives	Symptoms cleared with Nystatin.
<i>T. glabrata</i>	vagina	vaginal discharge	-	Ampicillin for post-operative infection	Symptoms cleared with Nystatin.
<i>T. glabrata</i>	vagina	pruritus vulvae, discharge	-	On oral contraceptives	Symptoms cleared with Nystatin and Pimafucin.
<i>C. krusei</i>	vagina	vaginal discharge	-	-	Symptoms cleared with Nystatin.
<i>C. krusei</i>	vagina	vaginal discharge, inflammation and irritation	-	-	Symptoms cleared with Nystatin.
<i>C. parapsilosis</i>	ear	chronic infection	-	-	Cleared spontaneously.
<i>C. parapsilosis</i>	ear	otitis externa	-	-	Slow clearing of symptoms with Nystatin.
<i>C. parapsilosis</i>	vagina	pruritus vulvae and ani	-	-	Symptoms cleared with Nystatin and Pimafucin.
<i>C. parapsilosis</i>	ear	otorrhoea	-	oral tetracycline amphotericin B, erythromycin	Nystatin given but no response initially - patient did not return.
<i>C. parapsilosis</i>	skin ulcer	No	-	local anti-biotics	Condition improved with varicose vein treatment.

yeast-like fungi is impractical perhaps the preferred terminology for these organisms would be, "yeast-like fungi (not *C. albicans*)", when the germ-tube test is used as a screening method. It is clear that *Candida krusei*, the genus *Torulopsis*, and *T. glabrata* in particular, have clinical significance in thrush vaginitis. *Candida parapsilosis* is often implicated in external otitis. Further investigation may well show that other yeast-like fungi are also clinically significant.

Summary

A survey was made on the incidence of yeast-like fungi, other than *C. albicans*, in material sent for microbiological examination.

Clinical evaluation showed that these organisms can be significant and more accurate speciation is preferable to the commonly adopted practice of referring to them as "*Candida species*".

Acknowledgments

The author wishes to thank Dr J. T. O'Brien and Mr F. M. Rush-Munro, Mycologist

at the National Health Institute, for their helpful advice in the preparation of this paper.

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Two Isolations of Methicillin Resistant *Staphylococcus aureus*

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Received for publication, November 1975

Introduction

Methicillin resistant strains of *Staphylococcus aureus* were isolated first in England in 1960 by Jevons⁵. The incidence of these strains among hospital isolates of *Staphylococcus aureus* increased from 0.06 percent in 1960 to 4.11 percent in 1970⁶. Because of the existence of these strains overseas, all *Staphylococcus aureus* isolated in this laboratory have been routinely tested for methicillin sensitivity since 1970.

This paper reports two separate, apparently unconnected, isolations of methicillin resistant strains of *Staphylococcus aureus* at Auckland Hospital in April and August 1975. These are believed to be the first such isolates in New Zealand. The problems associated with detecting methicillin resistance are discussed.

Methods

1. Disc sensitivity test

The organism is picked from the original culture, and streaked on to 1/8 of a DNA plate and the remainder inoculated into 10 percent NaCl broth. The broths are incubated in a 37°C water bath for 4-5h. A loopful of each broth culture is then removed and streaked across an "Oxoid" Sensitest agar plate. A filter paper strip of methicillin prepared by Alpha Biologicals Ltd, Auckland, is placed on the plate, at right angles to the inocula, and the plate incubated at 30°C overnight. Up to five cultures plus a control may be tested on one plate. Methicillin sensitive staphylococci give a clear zone of inhibition near the paper strip. Any organisms showing resistance, or reduced sensitivity have a tube dilution MIC done.

2. Tube dilution MIC

Preparation of antibiotic concentrations

Two rows of 12 sterile test tubes are set up. Doubling dilutions of methicillin in Penassay broth are prepared in duplicate, ranging in concentration from 25 µg/ml to 0.05 µg/ml. The aliquot removed from tube 10 is transferred to tube 11 (sterility control) and Penassay broth alone added to tube 12 (organism control).

Preparation of Inoculum

Overnight broth cultures of the control (Oxford *Staphylococcus*) and test organisms

are diluted to a turbidity to match a Wellcome opacity tube 1. A further 1:10 dilution of this suspension is made to give the working inoculum. With a sterile pasteur pipette delivering 0.025 ml/drop, one drop of working inoculum of the test organism is added to each tube of row 1, omitting tube 11. The control organism is similarly inoculated to row 2. The test is incubated at 30°C for 48 hours. The MIC is the reciprocal of the last tube to show no growth.

Case 1

Mr J, a 46-year-old alcoholic had his arm amputated below the elbow, and this stump subsequently became infected with *Staphylococcus aureus*. The organism was resistant to penicillin, tetracycline, erythromycin, lincomycin, cotrimoxazole and methicillin by disc diffusion tests. It was sensitive to vancomycin, fucidin and gentamicin. The MIC to methicillin was 25 µg/ml. In spite of vigorous treatment for five weeks with gentamicin and topical fucidin, the organism could not be cleared from the stump. This was thought to be largely due to the fact that the blood supply to the area was very poor. As the patient was suffering some pain from the stump, the arm was re-amputated above the elbow to clean tissue and the patient has since recovered. No cross infection occurred. Barrier nursing was employed throughout and special precautions taken when disposing of any material from the patient. The organism was not isolated from swabs taken from various other sites on the patient. The source of the infection is therefore unknown.

Case 2

Mr S, an 18-year-old male was involved in a RTA in Suva. He was admitted to Auckland Hospital because of head injuries and in August, approximately one month after admission, a methicillin resistant *Staphylococcus aureus* was isolated from his tracheal aspirate. The organism was also isolated from the tracheostomy site and the suction tube but was not considered to be clinically significant. No specific treatment was instituted. The organism is still being intermittently isolated

from the patient (October 1975) without any autogenous spread. It was resistant to penicillin, erythromycin, cotrimoxazole, tetracycline and methicillin by disc diffusion tests. It was sensitive to lincomycin, cephalosporin, gentamicin, kanamycin and chloramphenicol. The MIC to methicillin was 25 µg/ml. No cross infection occurred and swabs taken from multiple sites on the patient were negative.

Discussion

It is now well established that there are difficulties in methicillin sensitivity testing. This is due to the "heterogeneous" nature of the resistance in some 20-25 percent of methicillin resistant *Staphylococcus aureus*. When testing these organisms, only a small minority of cells will appear highly resistant and these will also grow relatively slowly in the presence of methicillin. As a result, if the sensitivity test is carried out on ordinary media at 37°C a resistant culture may appear sensitive.

Several methods have been devised to overcome the problem. Barber² showed that the addition of 5 percent salt to the test agar allowed the disc test to be carried out overnight at 37°C. This is generally reliable but some batches of methicillin salt agar have been found to be inhibitory to resistant staphylococci. Annear¹ showed that all methicillin resistant staphylococci could be recognised by overnight incubation at 30°C. The lower temperature enhances the resistance of the "heterogeneous" cultures and has no effect on sensitive strains.

Cloxacillin, rather than methicillin is the antibiotic used clinically in this hospital for

penicillinase-producing *Staphylococcus aureus* infections. Methicillin, however, should always be used as the antibiotic for *in vitro* testing because diffusion tests with cloxacillin do not always reveal resistance³. There is complete cross resistance and cross sensitivity between the antibiotics.

It is also important that inoculum strength should be adequate. If too light an inoculum is used the small number of highly resistant organisms may be missed. Methicillin, like all the penicillins, is unstable and may degrade on storage. It is best stored in the deep freeze, preferably with a desiccant. The detection of methicillin resistance is well reviewed by Hewitt, Coe and Parker⁴.

Conclusion

Two isolations of methicillin resistant *Staphylococcus aureus* are reported. The importance of following correct procedures for methicillin sensitivity testing is stressed so that any further isolates will be recognised. This will ensure that alternative antimicrobial therapy is promptly instituted and will minimise any dissemination of these organisms in the environment.

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Effect of Saline on Survival of Micro-organisms from Bronchial Aspirates

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Received for publication, March 1976

Introduction

Diagnosis of lower respiratory tract infection is often made using bronchial secretions aspirated by catheter. If there is insufficient quantity for aspiration, saline is introduced into the bronchial space to provide a vehicle for the secretions. As a result saline often becomes the major constituent of bronchial aspirate specimens received by the laboratory.

Rein *et al.* (1973)² examined the effect of saline on bacteria commonly isolated from lower respiratory passages. They concluded that when specimens are inoculated onto culture media with minimum delay, sterile saline free of bacteriostatic agents may be satisfactory. However, we have made further tests to assess the effect of saline solutions used at Green Lane Hospital on bronchial micro-organisms

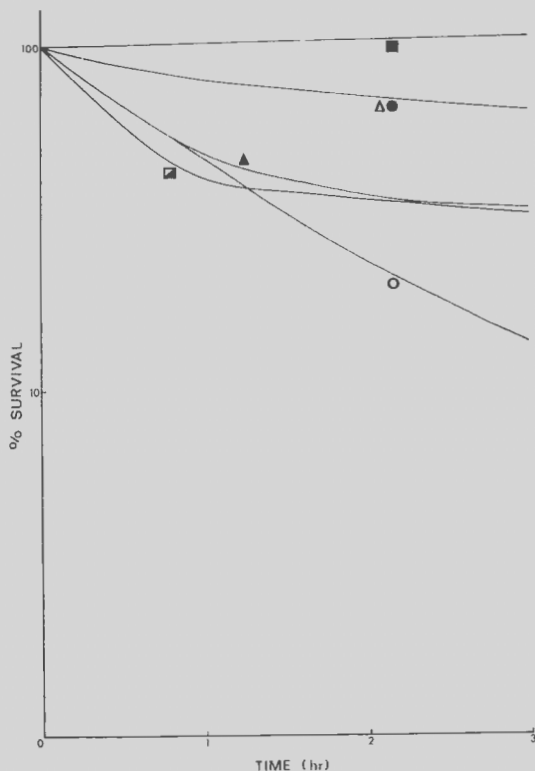


Figure 1: Survival of *E. coli* (Δ), *Klebsiella* spp. (●), *Staphylococcus aureus* (▲), *Streptococcus viridans* (○), *Candida albicans* (■) and *Aspergillus fumigatus* (◻) in saline (—) solutions.

and have been unable to confirm their conclusion. Survival in lactated Ringers solution was also examined. Lactated Ringers solution was recommended as an alternative to saline by Rein *et al.* (1973)².

Materials and Methods

The conditions that bronchial washings are subject to in practice were duplicated as closely as possible in this experimental work.

Micro-organisms in the logarithmic phase of growth were suspended at a concentration of $10^4 - 10^5$ viable units per ml in 10 ml of each test solution and incubated at 22°C (room temperature). At intervals of 0, $\frac{1}{2}$, 1, 2 and 3 hours samples were removed and diluted with saline. Plate counts were made by the "Surface Viable Count by Spreading Method" of Cruikshank *et al.* (1975)¹.

Survival was calculated as percentage of original population viable at a given time.

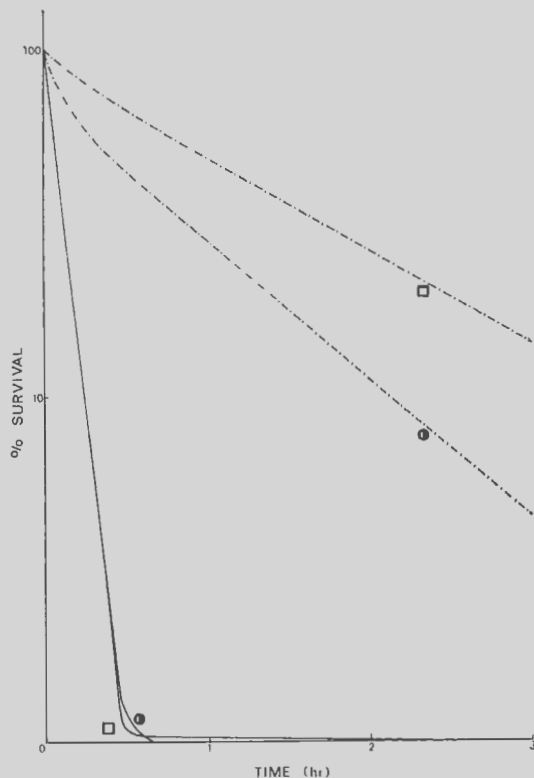


Figure 2: Survival of *Haemophilus influenzae* (◻) and *Streptococcus pneumoniae* (●) in saline (—) and Lactated Ringers (---) solutions.

All bacteria tested were recent isolates from respiratory specimens. Five isolates each of *Staphylococcus aureus*, *Streptococcus viridans*, *Streptococcus pneumoniae*, *Escherichia coli*, *Klebsiella* spp., *Haemophilus influenzae*; two each of *Candida albicans*, and one of *Aspergillus fumigatus* were tested in saline. Two isolates each of *H. influenzae* and *Strep. pneumoniae* were tested in lactated Ringers solution.

Sterile, non-intravenous 0.9 percent saline was supplied by Hospital Solutions Unit. Lactated Ringers solution was supplied by McGaw Ethicals Ltd. as Lactated Ringers Injection (Hartmann's).

A. fumigatus and *C. albicans* samples were cultured on Sabdex agar aerobically. *Streptococcus* spp. and *H. influenzae* were cultured on blood and chocolate agar incubated in candle jars. All other micro-organisms were cultured on blood agar aerobically. All cultures were incubated at 37°C.

Percentage survival of each microbial population was calculated from colony counts and plotted against time on semilog paper.

Results

The percentage survival of different microorganisms in various solutions is shown in Figures 1 and 2.

Discussion

Saline killed 99.9 percent *H. influenzae* and 99.6 percent *Strep. pneumoniae* within half an hour. As these are the most common respiratory pathogens isolated in this department, sterile saline would appear to be an unsuitable vehicle for bronchial secretions even if immediate culture were possible.

When lactated Ringers solution was used instead of saline, survival of *H. influenzae* and *Strep. pneumoniae* was greatly increased. At half an hour survival changed from 0.1 percent to 66.3 percent for *H. influenzae* and from 0.4 percent to 39.7 percent for *Strep. pneumoniae*.

Rein *et al.* (1973)² also showed increases in survival of micro-organisms in lactated Ringers solution compared to saline. However,

on comparison with their results we obtained markedly lower percentages survival in saline for *H. influenzae*, *Strep. pneumoniae*, *Staph. aureus* and *Strep. viridans*.

This may be due to strain variation within a species. Rein *et al.* (1973)² used one strain in duplicate or triplicate. We tested seven strains each of *H. influenzae* and *Strep. pneumoniae* and five each of *Staph. aureus* and *Strep. viridans*.

Our results demonstrate that survival of major, common pathogens of the respiratory tract in sterile saline is unsatisfactory. When recovery of pathogens from bronchial aspirates is required a balanced salt solution, such as lactated Ringers, should be used and cultures made as soon as possible after collection.

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An Evaluation of a Rapid Coombs Technique

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Based on a paper read at NZIMLT Conference 1975, Palmerston North

Received for publication, October 1975

Summary

It is well known that several factors influence the antigen-antibody reaction, e.g., pH, temperature, time, and ionic strength of the red cell suspension medium^{1, 2, 3, 4}. If 0.85 percent saline as the red cell suspension medium is substituted with salt solution of low ionic strength, this permits a considerable decrease in the incubation time which is of practical importance in urgent laboratory work. The sensitivity of the reaction does not appear to be decreased by this procedure. In many instances an increase in reaction strength can be observed.

Introduction

Low and Messeter⁵ have published an article showing that incubation time for the indirect Coombs method can be reduced to five minutes if 0.85 percent saline is substituted with a Low Ionic Strength Salt Solution (LISS) as the

final suspending medium for red cells. This brief evaluation of their method is not intended to compare it with other rapid or conventional methods in use, but simply to show advantages or disadvantages found with this method in a routine blood bank laboratory when used as an alternative method of antibody detection. Experimentation with differing ionic strengths was not undertaken as Low and Messeter had done, instead their findings that a 0.03 Molar solution was the most suitable was evaluated to determine its usefulness in the working laboratory.

Materials

Low Ionic Strength Solution (LISS)

(Sodium Chloride Molarity, 0.03 M)

1. Saline 0.17 M 180 ml
2. Phosphate Buffer 0.15 M pH 6.7 20 ml
3. Sodium Glycinate 0.3 M pH 6.7 800 ml

Sera and Red Blood Cells. All serum dilutions were made in 0.85 percent saline. Red cells were washed in 0.85 percent saline and 3-5 percent suspensions in LISS or 0.85 percent saline used.

Antiglobulin sera — Ortho anti-human globulin was used throughout but others, namely Burroughs-Wellcome, Lee, Gamma Biotest, Dade, Biolab and Behringwerke green contrast sera have been used in this study with similar results. Kallstad anti-human C₃ was used in assessing complement fixation at Low Ionic Strength.

The first problem encountered was that none of the normal chemical suppliers could provide sodium glycinate and indicated a delay of three to four months was involved. Glycine on the other hand was more readily available and after considering that the only difference between sodium glycinate and glycine was that one had a sodium ion attached and the other a hydrogen ion, we set about making up the Low Ionic Strength Solution using 0.3 Molar Glycine instead of 0.3 Molar Sodium Glycinate as used by Low and Messeter.

Method

1. Wash cells three times in 0.85 percent saline.
2. Resuspend to 3 percent in LISS instead of 0.85 percent saline after final wash.
3. Add one drop of LISS suspended cells to one drop of serum.
4. Incubate mixture at 37°C for five minutes.
5. Wash four times in 0.85 percent saline and add one drop of anti-human globulin.
6. Spin and read macroscopically.

N.B. Apart from steps two and four, the method is the same as for a normal Indirect Coombs Test.

Results

Results using this method were most encouraging after an initial disappointment when titres appeared to be the same or slightly lower than those by saline Coombs technique. The authors⁵ of the method claimed higher titres with LISS than saline Coombs. The cause was found to be our conventional saline Coombs method of *two* drops of serum to *one* drop of 3 percent cells in LISS. The ionic strength of the resultant mixture was being raised and somewhat negating its Low Ionic Strength effect. When using *one* drop of serum to *one* drop of LISS suspended cells, the titres went up by two to three steps in most cases.

Table I

Titration records of various antibody containing sera against cells suspended in LISS and Saline respectively.

ANTIBODY	LISS	SALINE
Anti D 1	1:128	1:128
Anti D 2	1:32	1:4
Anti D 3	1:1024	1:512
Anti D 4	1:128	1:64
Anti D 5	1:4	1:4
Anti C	1:64	1:32
Anti c	1:128	1:32
Anti ce	1:2	Nil
Anti CC ^w	1:8	1:4
Anti C ^w	1:8	1:4
Anti E	1:32	1:8
Anti e	1:32	1:16
Anti Fy ^a	1:128	1:128
Anti Fy ^b	1:32	1:8
Anti Jk ^a	1:2	1:2
Anti Jk ^b	1:32	1:32
Anti Kell	1:32	1:32
Anti Cellano	1:8	1:8
Anti Le ^a	1:1	1:1
Anti Le ^b	1:16	1:8
Aiti S	1:32	1:4
Anti s	1:64	1:32
Anti M	1:512	1:256
Anti N	1:2	1:2
Anti Co ^b	1:16	1:1

Examples of these titres are shown in Table 1. Some are antisera in use in our laboratory, others are sera from patients with previously identified antibodies.

Mollison *et al.*⁶ has stated that a limitation in using a Low Ionic Strength medium in demonstrating antibodies is the occurrence of false positive results; at Low Ionic Strength, normal immunoglobulin undergoes a change, probably aggregation, which results in the fixing of complement components to the red cell surface. When the cells are washed at normal ionic strength, the immunoglobulin but not the complement is removed and the red cells are therefore agglutinated by anti-complement reagents. All sera shown in Table 1 were tested by the LISS method against a commercial cell panel of ten cells typed for all common antigens and eight of our fully typed staff panel. None showed another specificity other than that depicted and none gave any false positives. At the same time similar cell panels suspended in LISS were

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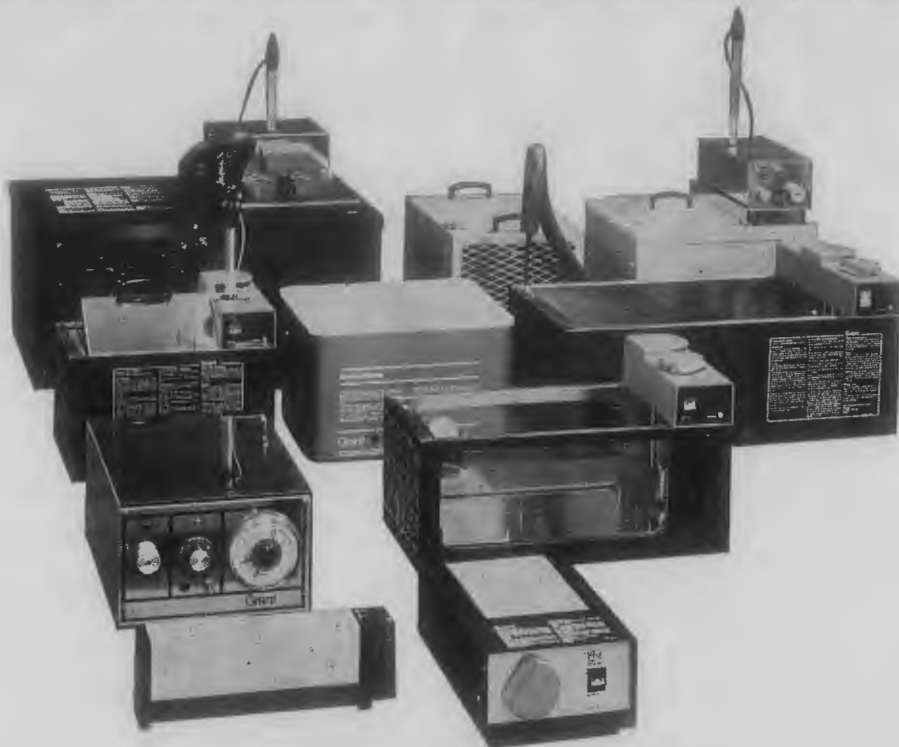
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incubated for one hour and no false positive or negative reactions occurred after this extended incubation time, except when a goat anti-complement reagent was used instead of a broad spectrum Coombs reagent. In this case agglutination occurred with all cells tested showing the effect of complement fixation at Low Ionic Strength. As this did not occur with any broad spectrum Coombs reagent tested, it was not considered a problem for routine use but must be borne in mind when immunoglobulin typing an antibody using cells suspended in LISS.

Rosenfield⁷ has stated; that some antibodies react poorly by Low Ionic Strength methods. (Notably some Duffy and Kidd antibodies.) As can be seen from the titres shown all sera tested reacted with titres as high by LISS as by saline Coombs methods. In fact a more definite end point was evident in most cases even though the titres were similar, the end reactions were stronger and therefore easier to determine.

Discussion

Although not in routine use as yet in our laboratory, this method has been used in parallel with our routine antibody screening methods for five months now and has not shown any false positives and has not missed any antibodies detectable by conventional methods except for one anti Le^a which reacted by a two stage ficin technique only and not at all by any other method, enzyme, Coombs or LISS.

One antibody, an anti ce, gave very nebulous reactions by other methods tried but gave good visual reactions when LISS was used and enabled identification of an otherwise unidentifiable antibody.

LISS has been used on many occasions now in our laboratory to rapidly screen donors for compatibility with patients with high incidence or multiple antibodies. In each case, blood found compatible by this preliminary screening has proven to be compatible when fully crossmatched by our saline, Low's papain, and albumin-Coombs crossmatch techniques and has been transfused without any untoward patient reaction.

Elutions both in 0.85 percent saline and AB serum tested by LISS have shown higher titres and greater avidity than by saline Coombs and this has proven to be most useful when

the antibody coating on cord cells of infants with Haemolytic Disease of the Newborn is in question. So far Immune Anti A, Immune Anti B, Anti D and Anti E have been demonstrated by this technique on cord cells in our laboratory.

The clinical importance of a reduced incubation time for crossmatching in urgent cases cannot be minimised and it is in this aspect of laboratory work more than any other that LISS will have its greatest impact. In our laboratory it has been used in parallel on some routine crossmatches and on ten occasions to date in emergency crossmatching. Although we have yet to detect any incompatibilities by the LISS method or by our other methods used in these emergency crossmatches, it is reassuring to be able to issue blood under emergency conditions knowing that a Coombs method has been completed and found to be compatible.

Samples of the three batches of LISS which have been made up in our laboratory have been retained and retired against a control anti D each month to test the reagents stability over a period of five months and have achieved the same titre, that is 256 each time. Although this is only a limited study of its storage life it does seem to indicate that after millipore filtering the reagent is stable for what would be the normal expected working life of most serology reagents in routine use.

To sum up, this is a method which has been used routinely by Low and Messeter⁵ in Lund University, Sweden, for over six years and with it they have transfused more than 100,000 units of blood without any antibody antigen transfusion reactions occurring. Our limited study has shown it to be ideal as a complementary method to others in existence in the routine laboratory with its greatest disadvantage being that it requires more diligence to make up than 0.85 percent saline, but no more than any other buffered salt solution, and its greatest value lying in its rapidity, simplicity, and sensitivity.

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Effect of Storage Time and Temperature on some Haematological Parameters

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Received for publication, January 1976

Summary

Blood collected into EDTA was subjected to storage at 4°C and room temperature and tested at intervals up to 48h for various haematologic parameters. Using narrow acceptance limits it is suggested that accurate results can be obtained for haemoglobin, white cell count and mean cell volume up to 24h after collection with storage at either 4°C or room temperature.

Platelets stored at either 4°C or room temperature should be counted within 6h of collection.

Introduction

“One of the advantages of EDTA as the anticoagulant of choice for cell counting and sizing is that it does not significantly alter the cells over an extended period of time.”

Statements of this nature appear regularly in text books and are handed down from technologist to technologist without further ado.

Occasionally an event occurs that causes one to wonder if this is correct or not but usually the pressure of work pushes it out of our minds.

Staff from the laboratory were to be involved in a study requiring large numbers of blood specimens to be airfreighted from overseas and consequently any changes that may occur could have been of great significance.

Materials and Methods

A total of 30 blood samples from six adults were collected into Venoject Vacutainers containing 1.5 mg/ml Sodium EDTA. Haematological parameters were obtained from the Coulter Model S and platelet counts from the Technicon Autocounter. Each specimen was tested at 0, 2, 4, 6 and 8 hours and then at 24, 36 and 48h.

Room temperature observations were made at 23°C.

All results were ultimately converted to a percentage of the “Zero time” result.

Ninety-five percent confidence limits are shown; these figures were calculated from internal quality control information, and were as follows:

Haemoglobin — plus or minus 2 percent of the zero time value.

Mean Cell Volume — plus or minus 2 percent of the zero time value.

Platelets — plus or minus 6 percent of the zero time value.

White Cell Counts — plus or minus 8 percent of the zero time value.

Results

Haemoglobin Estimation — (Figure 1).

Of 113 results two fell outside the acceptance limits of ± 2 percent.

One result was 3 percent outside at 8h, the other at 36h was also 3 percent from zero time. These changes may well represent random distribution and may be of little consequence.

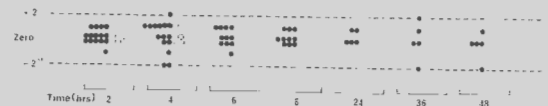


Figure 1.—Change in haemoglobin level over 48 hours.
● = 4°C ○ = 23°C

Mean Cell Volume — (Figure 2)

Of 113 results three fell outside the acceptance limits of ± 2 percent. No results were outside ± 3 percent of the zero time result.

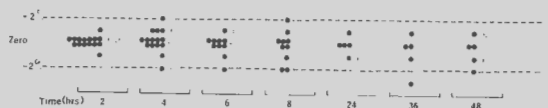


Figure 2.—Change in mean cell volume over 48 hours.
● = 4°C ○ = 23°C

White Cell Count — (Figure 3)

Distribution was within the acceptance limits of ± 8 percent up to the 24h testing time.

After 24h the distribution widened with all results outside these limits falling below the lower 8 percent limit. Generally the white count results started falling below the zero time value at 8h with this trend continuing until 48h but at the same time the distribution widened greatly.

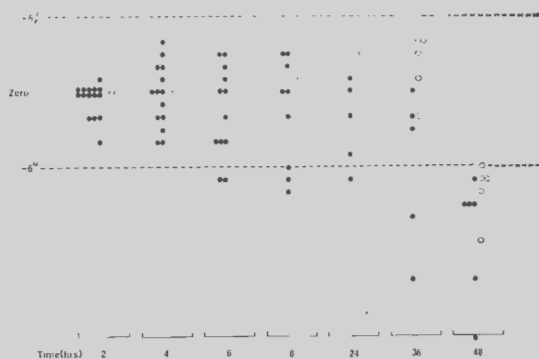


Figure 3.—Change in white cell count over 48 hours.
● = 4°C ○ = 23°C

Platelet Count — (Figure 4)

Distribution was within the acceptance limits of ± 6 percent until the 6h counts when two of the figures fell below this line.

This pattern remained until the 36h count

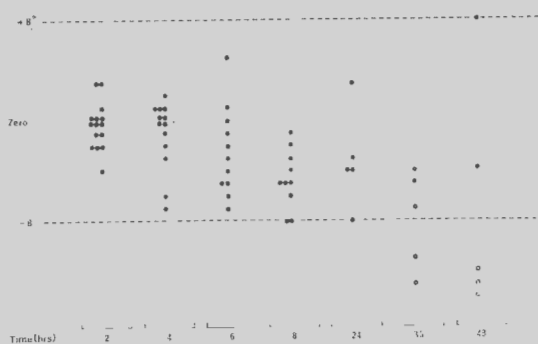


Figure 4.—Change in platelet count over 48 hours.
● = 4°C ○ = 23°C

when these figures dropped significantly lower.

At 48h the entire group of figures were at or greatly below the lower 6 percent confidence limit.

Discussion

The published data covering the effects of

storage of blood collected into EDTA usually cover only two time points — 12 and 24h. Dacie² states that storage changes can be minimised by holding the bloods at 4°C but indicates that platelets, white cells and PCV should be estimated within 6h.

Gagon *et al.*³ tested only white cells and showed at either room temperature or 8°C the white cell count was still acceptable at 24h. Lampasso⁴ on the other hand extends the time limit to 48h at 4°C but only to 24h at 23°C.

Neither Dacie nor Gagon offer any indications of what their acceptance limits were so it is difficult to judge what they regard as significant. Our experience would suggest that by 8h the white cell count is starting to drift down and that by 24h, although still generally within the limits, the value of a given figure is becoming open to question. Certainly by 36 and 48h the figures may vary to such an extent as to be of little value particularly if trying to relate any given result to past results, e.g., when following progress during intensive chemotherapy, etc.

Dacie states that platelets should be counted within 6h if stored at 4°C while Gagon allows storage at room temperature for up to 5h before effects are noted. On the other hand Lampasso would accept storage for up to 24h at 4°C before significant changes occur. Our series would agree more closely with Dacie and Gagon in that 12 percent of our results fell outside ± 6 percent at 6, 8 and 24h.

Within these time periods no result was greater than 8 percent from the zero count. At 36h the scatter of results had widened with results falling above and below the limits while at 48h 90 percent of the results were significantly below the lower 6 percent limit.

Prior to the introduction of automated equipment of the Coulter S type into this country, the MCV was a rarely calculated parameter. With the spread of Coulter S's and similar sophisticated counting devices through the larger laboratories, the preservation of the MCV assumes a new importance particularly in so far as it is a directly measured parameter and is required in the calculation of the MCH, MCHC and PCV. In our situation it assumed considerable diagnostic significance and was therefore included in this study. Limits of ± 2 percent were accepted and up to 24h only one point had fallen from this range.

This change was also noted at 36-48h.

None of the out of limit results was more than ± 3 percent of the zero count. These 24h findings are in keeping with those reported by Lawrence *et al.*⁵ and Brittin *et al.*¹. It was inferred in Lawrence's study that the MCV may be able to be accurately measured beyond 24h. As indicated by our results (maximum variation ± 3 percent) it seems that up to 48h storage at 4°C may still yield an acceptable result.

The estimation of haemoglobin is (along with total white counts) one of the most commonly asked for measurements.

Lampasso and Gagon both allow re-estimation to occur up to 48h after collection with storage at either 4°C or room temperature. The 4°C findings are confirmed at the 24h level by Brittin *et al.* Dacie, however, infers that this measurement may be made up to "days" later with no real loss of accuracy. We have set an acceptance limit of ± 2 percent of the zero time figure. Our acceptance limits when compared with those given by Lampasso are much narrower and it is likely that this reflects the use of a Coulter Model S on our part against his manual techniques. It was thought that changing specimens from one storage temperature to another, e.g., 4°C to room

temperature or vice versa, may alter the final result. As indicated earlier various combinations of these factors have been tried.

No appreciable change in the result was noted.

Acknowledgments

Our thanks to Mr A. Nixon for his continuing help and encouragement.

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An enquiry about the diminishing numbers of specimens examined in the latter stages of the investigation elicited the following information from the authors. The experiment was initially designed to simulate the conditions of airfreighting blood from Fiji. In order to extend the testing to 48h substantial volumes of blood had to be obtained from volunteers and these could not always be obtained. However, results during the first 8h period seemed more important and relevant to the normal laboratory situation and the numbers only decreased after this time.—Editor.

An Assessment of the Radiometer ABL 1

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Received for publication, November 1975

Based on a paper read at the NZIMLT Conference, Palmerston North, 1975

Introduction

Demand for equipment that provides for rapid, simple and accurate acid-base analysis of blood has been increasing steadily since 1954 when Dr P. Astrup first developed the equilibration technique, a simple technique involving 3 pH measurements. The first direct measurement of partial pressure of oxygen was developed by Clark two years later, thereby permitting the measurement of pO₂ and the calculation of oxygen saturation. These remained the only analyses available until 1958 when Severinghaus modified the pCO₂ membrane electrode of Stow which provided a direct method for complete acid-base determinations. These were manual methods, the accuracy of calibrations and measurements being dependent on the skill of the operator.

Most equipment in this field is used 24 hours per day and operated by a variety of laboratory personnel, some without adequate training or experience. The makers of blood gas instruments realised the need to automate and Radiometer feel they have the answer in the ABL 1. This instrument was the first fully automated blood-gas analyser available in this country. Some of the success of this instrument may be attributed to this fact, but production increases of 300 percent per annum indicate its international acceptance.

Description

The features of the apparatus include:

1. Self-calibration.
2. Self-generation of calibrating solutions with known gas tension.

3. Automatic measurement of, and correction for barometric pressure.
4. Measurement of haemoglobin concentration.
5. Automatic flushing and rinsing.
6. Built-in microcomputer for control and accuracy.
7. Hard copy printout of results with facility for omission of unwanted parameters.

The instrument itself is divided into three sections.

- A. The gas supply section.
- B. The electronic section.
- C. The wet section.

A. *The gas supply section*

This section supplies the gases required for the equilibration of the calibrating solutions and operates on the Poiseuille law adapted to gases which states "that the flow of gas passing a capillary is inversely proportional to the frictional resistance of the tube and to the viscosity of the gas."

If the gas flow is laminar and the pressure drop along the tube is maintained, the gas flow depends on the viscosity of the gas.

Two gases are required, pure carbon dioxide from a pressurised cylinder and atmospheric air. The normal carbon dioxide content of air is 0.03 percent. The value can be slightly higher in areas where people are working. Radiometer have assumed that for this instrument the carbon dioxide content of air is 0.04 percent.

The gas mixer in this section of the instrument provides gases of known concentrations which are nominally 5.61 percent and 11.22 percent CO₂ in air. These values vary slightly for each machine. Individual instruments are therefore factory standardised for their own gas mixers.

Analysis of the gas mixtures from the machine at Green Lane Hospital by Haldane apparatus showed a difference of 0.02 percent. The gases were 5.64 percent and 11.25 percent CO₂ in air instead of 5.62 and 11.23 percent respectively.

Since the gas mixer has a stated accuracy of 0.08 percent the above results are well within the acceptable limits.

B. *The electronic section*

This section has six measuring channels, one each for pH, pCO₂, pO₂, haemoglobin, barometric pressure and gas supply unit. The signals from these channels are fed to a

microcomputer which is provided with a programme library and memory. This programme library controls the operation of the machine while the memory stores the results from the previous calibration. These calibration values will remain in the memory unit until replaced by new ones from a subsequent acceptable calibration. If a calibration is unacceptable for any reason the old values will remain in the memory and the print-out of new calibration will be furnished with question marks indicating its unacceptability. The presence of question marks does not necessarily mean the results themselves are wrong, but that something requires attention, e.g., if the rinse saline reservoir was empty the "rinse" light would flash and any subsequent analysis would have question marks. Before any results are printed out the computer checks

- (a) The liquid sensors in the wet section.
- (b) That the gas supply is sufficient.
- (c) The temperature.

If any of these are not right question marks will appear on the print-out. The question marks may also be due to erroneous results. Correction of drift and other errors in calibrations are done by the computer immediately after the print-out.

The instruction manual supplied with each instrument suggests turning the power off for 15 seconds daily, an operation which clears the memory in the micro-computer and initiates a special "start-up" calibration which checks out electrode sensitivity. If the routine two-hourly calibrations are acceptable this only needs to be done when the operator is worried about the electrodes or their results. The "start-up" calibration can be done without turning the power off by earthing a particular point in the electronic section. This procedure is better demonstrated by the agent than described here. If it is at all possible the power should be left on because the pO₂ electrode draws its polarising voltage from the mains supply and if turned off takes approximately 24 hours to stabilise.

The instrument at Green Lane Hospital is left on continuously. An electrode sensitivity check or "start-up" calibration is done if the routine two-hourly calibrations indicate something is wrong and any electrode membrane changes are done without turning the power off.

C. The wet section

This is an air-thermostated chamber housing all the necessary electrodes and calibrating solutions. As this machine calibrates on liquid there is no need for a blood-gas factor. Independent heaters are used for the calibrating liquids to ensure adequate temperature control. Heat exchangers are also present in this section to ensure all liquid entering the measuring chamber are at 37°C. These liquids, blood, saline and calibrating solutions all enter the measuring chamber in the same well-defined manner thereby completely eliminating any errors resulting from operator technique.

Experimental Findings

To demonstrate operator technique (Table I) a large sample of blood was split into 15 equal size aliquots which were kept iced. Fifteen operators each took a sample and measured the different parameters on the ABL 1 and BMS 3.

Table I
OPERATOR TECHNIQUE

		ABL 1	BMS 3
pH	Mean	7.300	7.306
	S.D.	0.006	0.015
pCO ₂	Mean	40.7	37.6
	S.D.	0.8	2.4
pO ₂	Mean	182.6	164.3
	S.D.	2.5	11.7

The lower value and the larger error for pO₂ on the BMS 3 is a result of reading the result too soon, i.e., before the meter reading had reached its true peak.

Calibration stability is demonstrated in Table II. For convenience this was done in two groups.

GROUP A. A total of 25 consecutive 2-hourly calibrations done over a 48hr period.

GROUP B. Again, 25 consecutive calibrations done "on demand". This was done over a three to four-hour period by pressing the "calibrate" button whenever the machine was "ready". Repeated calibrations "on demand" are not recommended by the manufacturer as incoming buffer may not be completely equilibrated with the gas, but results obtained indicate no error.

The "repeatability" of the machine was investigated (Table III), by analysing a large sample of blood 20 times by various operators. The blood was stored on ice between each analysis.

Table II
CALIBRATION

	Buffer 1		Buffer 2		
	pH	pCO ₂	pH	pCO ₂	pO ₂
A. 2-hourly					
Mean	7.401	38.1	6.854	75.9	130.7
S.D.	0.001	0.5	0.001	0.7	0.7
C.V.	0.017	1.2	0.015	1.0	0.5
B. On demand					
Mean	7.400	38.8	6.854	77.5	130.4
S.D.	0.001	0.1	0.001	0.2	0.4
C.V.	0.016	0.3	0.015	0.3	0.3

Table III
PRECISION
(20 analyses)

	pH	pCO ₂	pO ₂	Hb
Mean	7.250	47.2	138.0	9.9
S.D.	0.001	0.2	1.0	0.1
C.V.	0.014	0.5	0.7	1.0
Specs.	0.003	1.0%	1.0%	1.0g%

All measured parameters are within the manufacturer's specifications. The haemoglobin value is just within the limits, but it is primarily measured for the calculated parameters. In practice, haemoglobin is reported in this laboratory and following an instrument adjustment on delivery, results compare favourably with those reported by the haematology department.

Regional Comparison

This was done, after an enforced clean, to check out our instrument.

Sufficient blood was collected and aliquoted anaerobically to small glass syringes which were sealed and then packed in ice. Each iced aliquot was then sent to a laboratory for measurement. To eliminate any errors due to time, all laboratories performed the analyses at a predetermined time.

The results obtained are from 10 different instruments in the four major hospitals in the city (Table IV).

Table IV
REGIONAL COMPARISON

	ABL 1	MANUAL	TOTAL
pH	Mean	7.336	7.350
	S.D.	0.009	0.024
pCO ₂	Mean	33.4	32.2
	S.D.	1.2	3.5
pO ₂	Mean	173.3	161.5
	S.D.	3.8	10.5

Four ABL 1's took part, the results of which are shown in Column 1. The manual instruments were of different make and these results

are shown in Column 2. The overall picture of all the instruments is shown in Column 3.

The overall result was very pleasing and all laboratories taking part expressed a desire to repeat the exercise regularly as a Quality Control procedure.

After 12 months of operation, the instrument at Green Lane Hospital has performed 8,000 analyses with a maximum of 60 per day. During this time there have been six major service calls which are as follows:

1. Haemoglobin reading of 99.9. This was due to a build-up of fibrin clots in the photometer. The machine was rendered inoperative because we could not get a satisfactory "start-up" calibration.

2. Results produced did not match the clinical state of the patient. This was also due to build-up of protein and fibrin in the instrument. After being stripped and cleaned, the instrument was re-evaluated.

These two "shut-downs" have been the only major faults we have had with the instrument and they both occurred before the "Cleaning Solution" produced by Radiometer was available. Problems of this type have disappeared since the regular use of the cleaning solution.

The other service calls have been for relatively minor things:

1. A blown capacitor in the pCO₂ amplifier board.

2. A lamp failure in the top liquid sensor.

3. A split hose to the gas mixer. This fault was peculiar to our instrument as later models used a different material for this tubing because the earlier material split. The instrument at Green Lane has now been modified.

4. An electronic fault in the Printer Board.

Looking back over the first year of operation, the most obvious item of importance is

cleanliness of the instrument. Regular use of the cleaning solution is important to avoid protein contamination in any form. A quick guide to the degree of contamination in the instrument can be obtained from a rising haemoglobin reading over a series of calibrations.

If the contamination gets too great it can be removed using toothpaste in a technique described by the manufacturers.

The instrument has performed well over the last 12 months, being used by a large number of different people. However, the BMS 3 system is still maintained for comparative studies and as a back-up if needed.

The capital cost of this instrument, the cost of necessary supplies and the availability of adequate service and spare parts, makes the use of manual instruments worthy of consideration in a laboratory where small numbers of analyses are performed. Against this, one would have to consider the availability of gas mixtures, the facilities of analysing these mixtures and the degree of skill of the operators.

The article mentions that question marks do not necessarily indicate that the results are wrong. V. versa results may be wrong without question marks occurring. This is usually due to a fibrin clot which is not always easy to remove. Critical assessment of the results cannot be neglected. Some operators find the haemoglobin result somewhat inaccurate but this may be due to the difficulty of mixing blood in a syringe. While most users of the ABL 1 would agree that it is an excellent machine when functioning properly, the purpose for which it is used makes electronic failure and delays in replacing boards a serious matter and an assurance that adequate spares are available is essential.—Editor.

Alpha-foetoprotein and its Use in Foetal Diagnosis: A Review

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Received for publication, February 1976

Introduction

The accessibility of amniotic fluid for analysis has widened the horizons of intrauterine diagnosis and the clinical management of the foetus. Cytological, immunological and

biochemical analyses from amniocentesis have changed the accepted therapeutic management of diseases and genetic defects. The discovery by Brock and Sutcliffe³ in 1972 that certain types of neural tube abnormalities showed a

marked rise in amniotic fluid alpha-foetoprotein levels (AFP), during the second trimester has added a new tool in the intrauterine diagnosis of foetal defects.

It is intended to discuss the use of AFP as a diagnostic technique in foetal medicine.

Source and Production

AFP is the major serum globulin of early foetal life². It is synthesised by the embryonic hepatocytes¹⁴, yolk sac¹⁷ and to a lesser extent the gut¹⁶, the liver being the principal site of production. Foetal serum levels of AFP are highest during 12 to 16 weeks' gestation², plateau between 22 to 32 weeks gestation, after which they rapidly decrease¹⁵. At term the AFP concentration undergoes further rapid decrease, dropping by 50 percent in the first five days of extrauterine life and in three days thereafter^{21, 23}.

AFP is also detectable in maternal blood from approximately 10 weeks' gestation¹⁴, the peak production being at 35 to 38 weeks' gestation⁴. This is thought to be of foetal origin¹⁸. However, maternal AFP levels would be expected to peak earlier in pregnancy if the source was solely foetal. Placental production of AFP was excluded by Abe (1970)¹; using a fluorescent antibody technique he could not demonstrate any AFP producing cells in the placenta.

Pitot and Heidelburger (1963)³¹ have explained the mechanism of AFP synthesis in adults using the repressor and operator gene hypothesis of Jacob and Monod²⁰. Although this is accepted by a number of workers, it by no means explains completely the synthesis of AFP in the pregnant woman.

Structure

AFP is a glycoprotein consisting of a single polypeptide chain with a molecular weight of 70,000³⁴. Early work on alpha-foetoprotein structure indicated a similarity to calf fetuin but more recent work^{22, 29, 34} has demonstrated that calf fetuin and human AFP are probably unrelated proteins. Nishi (1970)²⁸ demonstrated that the AFP found in patients with hepatoma was structurally and immunologically identical to AFP found in the foetus.

Assay

There are five main techniques for assaying AFP. These are listed in Table I with their relative limits of sensitivity. It is important to ensure that the correct technique is employed

Technique	Limit of Sensitivity
Immunodiffusion	10 μ g/ml
Immuno-electrophoresis	1 μ g/ml
Haemagglutination inhibition	500 ng/ml
Immuno-electrophoresis and auto radiography	20 ng/ml
Radioimmunoassay	<1 ng/ml

Table I: Relative sensitivities of techniques used for alpha-foetoprotein analysis.

when assaying test material. Generally radioimmunoassay is the best technique to use as it is rapid and the most sensitive. However, a number of dilutions may be necessary for amniotic fluid and this can be a serious source of error. It is imperative that the technique used is not one where most results fall on or about the limit of sensitivity as this produces serious errors in analysis. Normal ranges must be established in individual laboratories as there are wide method variations depending on the assay system and the standards used.

Clinical Significance

Figures 1 and 2 record the results obtained in this laboratory over the last 12 months. Amniotic fluid levels of AFP decrease with advancing gestation while maternal serum levels increase. The most suitable time for screening at-risk patients is between 15 and 22 weeks' gestation; before and after this time, results for neural tube defects become extremely variable.

Gestational Age

There is an inverse relationship to advancing gestational age and amniotic fluid AFP concentrations (Figure 1)³⁹. Approximately 20 percent of women are unable to give the date of their last menstrual period, thereby making the calculation of the gestational age of the foetus difficult if not impossible. It should be borne in mind that perinatal mortality increases dramatically in postmature infants. The lecithin/sphingomyelin (L/S) ratio and creatinine assays are accurate assessments of foetal organ maturity, however, their predictive ability can be negated by diabetes, hypertension and toxemia. AFP is affected less by maternal complications and has a predictive accuracy of ± 2 weeks either side of the gestational mean.

Open Neural Tube Defects

With only rare exceptions all open neural

tube defects have a raised amniotic fluid AFP during 15 to 22 weeks. As can be seen in Figure 1 the values are so high that there is no difficulty in interpretation. In the majority of these cases maternal serum AFP would also be well above the normal range (Figure 2). Maternal serum AFP values, however, rapidly return to normal gestational levels and can be well within the normal range while amniotic fluid AFP values are extremely high. This may frequently be seen around 20 weeks' gestation.

Closed Neural Tube Defects

Results from closed neural tube defects are variable (Figures 1 and 2). In these cases amniotic fluid is more reliable than maternal serum. Even in very severe closed neural tube defects both the liquor and maternal serum AFP can be normal. One possible explanation as to the variability of the results could be the time when the neural tube defect actually closed, and the thickness of the skin over the closure. The true meningoceles which account for 5 percent of all cases of spina bifida cystica, and are usually covered by a thick membrane, will in all likelihood remain undetected.

Foetal Distress and Death

The biochemical detection of foetal distress and/or death before the event is an invaluable diagnostic technique. In Table II, results show maternal serum AFP results and the clinical foetal distress and/or death do not always produce elevated maternal serum AFP results. One case of a foetal death, not presented in Table II, peaked at a maternal serum AFP of 17,500 ng/ml. However, another case of intrauterine death, number 12 in Table II did not show any rise.

Rhesus Isoimmunisation

In Rhesus isoimmunisation when the foetus is mildly to moderately affected generally no difference in amniotic fluid AFP is observed. In severe Rhesus isoimmunisation when foetal distress is present, amniotic fluid AFP is raised. Maternal serum produces variable results. It is felt that, at present, it is difficult to assess the severity of Rhesus isoimmunisation on AFP results alone as it is difficult to differentiate between it and foetal distress. The most useful aids are the measurement of the OD 450 peak²⁵ and antibody quantitation¹². Further investigations into the use of AFP in Rhesus isoimmunisation are being undertaken at present.

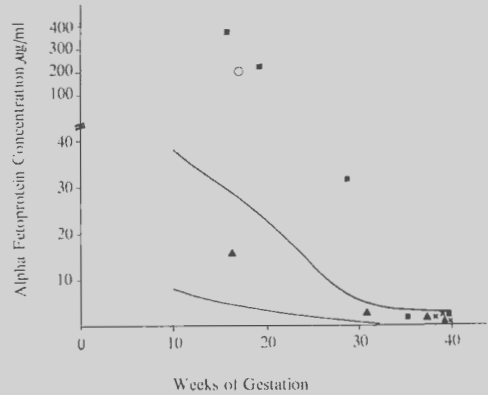


Figure 1. Amniotic fluid alpha-fetoprotein normal range (solid lines ± 2 SD) and results from neural tube defects. ■ = Anencephalic, O = Open Spina Bifida, ▲ = Closed Spina Bifida, x = Hydrocephalic.

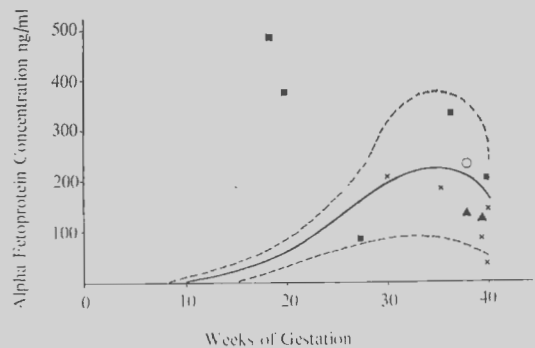


Figure 2. Maternal serum alpha-fetoprotein normal range and results from neural tube defects. ■ = Anencephalic, O = Open Spina Bifida, ▲ = Closed Spina Bifida, x = Hydrocephalic. Figure 2. (---- = ± 2 SD. — = mean).

Abortion

In women with threatened abortion, a high AFP for gestation is an unfavourable sign, however, normal AFP levels cannot be used to discriminate between viable and non-viable pregnancies^{13, 37}. Garaff and Seppala (1975)¹³, concluded that human placental lactogen was more reliable in helping to discriminate between normal and abnormal pregnancies in threatened abortion. They also described a case of a twin pregnancy where one foetus had died and the other was alive. Here, raised and normal amniotic fluid AFPs were found respectively¹³.

Patient number	Gestation in weeks	AFP ng/ml	Foetal heart irregularities	Meconium stained liquor	Intrauterine death
1	24	200	Yes	No	No
2	24	2400	No	No	Yes
3	27	1000	No	No	No
4	30	4000	Yes	Yes	Yes
5	33	70	Yes	No	No
6	35	800	Yes	Yes	No
7	35	520	No	No	Yes
8	38	185	Yes	No	No
9	38	205	Yes	No	No
10	40	700	Yes	No	No
11	40	850	No	No	No
12	40	178	No	No	Yes

Table II: Maternal serum alpha-foetoprotein results from 12 pregnancies where foetal distress and/or death were suspected.

Possible cause of error	False high result	False low result
Foetal blood contamination	Yes	No
Multiple pregnancies	Yes	No
Incorrect gestation	Yes	Yes
Inconsistent commercial standards	Yes	No
Maternal urine	No	Yes
Repeated freezing and thawing	No	Yes
Meconium	Yes	No
Severe toxæmia	No	Yes
Maternal diabetes	Yes	No

Table III: Factors which may cause discrepant alpha-foetoprotein results.

Multiple Pregnancies

In multiple pregnancies the maternal serum AFP is extremely high, in many instances levels comparable to those found in neural tube defects are obtained¹⁹. Amniotic fluid levels appear to be variable; frequently elevated.

Other Foetal Conditions

Raised AFP levels have also been reported in a number of other foetal conditions. To date these are: congenital nephrotic syndrome of the Finnish type³⁶, Turner's syndrome³⁵, omphalocele¹⁰, tetralogy of Fallot⁴², Rhesus isoimmunisation leading to intrauterine or neonatal death^{38, 44}, and congenital oesophageal atresia⁴⁰. Although the mechanism as to why AFP values should be raised in these conditions is not known, it is considered that the rise is associated with foetal distress rather than with the underlying disease.

Causes of False Results

Table III lists some of the common causes of false AFP results.

The presence of foetal blood in both amniotic fluid and maternal serum can produce a

substantial rise from the true AFP result. The AFP concentration of foetal blood is mg/ml and that in amniotic fluid and maternal serum is $\mu\text{g/ml}$ and ng/ml respectively, therefore, any contamination by foetal blood will produce raised levels of AFP. Milford Ward and Stewart (1974)²⁷ suggested screening amniotic fluids for foetal erythrocytes using the Kleihauer technique. In the author's laboratory all blood stained amniotic fluids are classed as unsuitable for AFP analysis and a repeat is requested. The amniotic fluids are examined spectrophotometrically³⁰ for the presence of haemoglobin F, this is for future reference. To date we have had no diagnostic amniotic fluids for AFP levels contaminated with foetal blood; this is entirely due to placental localisation under ultrasound prior to amniocentesis.

The significance of amniotic fluid and maternal serum AFP levels has been discussed earlier, however, it is essential to exclude multiple pregnancies when raised AFP's are discovered. Here again ultrasound can be a useful aid.

Grossly incorrect dates may well produce results which could be high or low, but when amended for the correct gestation, could be normal or raised. Brock (1974)⁵ cited two cases where the results were diagnostic of neural tube defects, but because of incorrect gestations were classed as normal.

Commercial AFP standards can produce variable high test results. This appears to be a function of the purity of the standards, as when the purity is increased and the protein contaminants decreased the AFP test results are lower and more consistent.

Reports of very low amniotic fluid AFP levels (less than 1 $\mu\text{g}/\text{ml}$) at 15 weeks) must be treated with suspicion as these samples are undoubtedly maternal urine specimens. The use of ultrasound prior to amniocentesis largely overcomes this problem. A creatinine and/or uric acid assay on the amniotic fluid will preclude maternal urine samples.

Constant freezing and thawing appears to form an AFP dimer which is immunologically unreactive³⁴. This problem requires further investigation.

Raised AFP values from meconium stained liquor probably results from foetal gastrointestinal tract secretions during development or periods of anoxia.

Patients with severe toxæmia tend to have lower serum, amniotic fluid and foetal serum AFP levels⁴¹. Seppala has suggested that it may be an indication of accelerated foetal liver maturation.

Diabetic pregnancies tend to have higher than normal AFP levels in maternal serum, amniotic fluid and foetal serum. In these cases Seppala has postulated retarded foetal liver maturation⁴¹.

Conclusion

AFP determinations are a valuable diagnostic aid in foetal medicine, particularly in the diagnosis of neural tube defects. The incidence of neural tube defects is 6.2 per 1,000 births in a British population, but there is considerable regional and racial variation⁶. The recurrence risks for parents who have had one or two affected children are about 5 percent and 10 to 12 percent respectively, and women who have had spina bifida surgery have a risk of 3 to 5 percent for having children with a neural tube defect^{7, 8}. Over 90 percent of infants with spina bifida are born to women who have not had previously affected children²⁶,

therefore cheap, rapid and reliable techniques for AFP are required in order to screen all pregnant women for foetal neural tube defects.

It is obvious, however, that there are conditions in which raised AFP's are present in either amniotic fluid, serum, or both, and a relatively unaffected foetus be delivered.

It is essential, therefore, that expertise in assaying and interpretation of AFP results is developed and that the assays should not be performed on a random basis. For a centre to assay for neural tube defects it should have the clinical facilities to back-up the laboratory results, i.e., skilled use of ultrasound and x-ray.

There is still a considerable amount of investigatory work to be done on AFP. Why should AFP appear in amniotic fluid and maternal serum in high concentrations in certain neural tube defects? Why does the maternal serum AFP rise when amniotic fluid AFP has a distinct fall with advancing gestation? A number of theories have been postulated to account for this phenomena, ranging from the Jacob Monod hypothesis^{20, 31} to an important component for the immunological maintenance of pregnancy^{24, 32, 33}. Why does AFP appear to be able to cross the placenta even in apparently normal pregnancies, despite its molecular weight of 70,000⁴³? Why do certain other foetal conditions produce raised AFP results which are comparable, in some cases, to those found in severe neural tube defects? What is the mechanism by which severe toxæmia and diabetic pregnancies are affected?

Clearly there is still a considerable gap in the diagnostic significance, the mechanism of action of AFP and the application of the results in foetal medicine. It is felt that, although AFP determinations are clinically useful they should not be undertaken as a general laboratory procedure as interpretation can be difficult.

Workers with AFP have reported therapeutic abortions on the basis of raised AFP results and a normal foetus being produced^{9, 11}.

Acknowledgment

I am most grateful to Mr Gavin Tisch for his help in assaying the AFP material.

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A Direct Automated Procedure for the Estimation of Serum Inorganic Phosphate

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Received for publication, March 1976

Summary

A direct method for the automated estimation of inorganic phosphate is presented. It involves the use of the usual reducing agents Metol (p-methyl ammonium sulphate), sodium metabisulphite with acid ammonium molybdate. Two additional reagents are used, a surfactant, sodium dodecasulphate which keeps normal proteins in solution and triethanolamine an organic base which intensifies the

colour and improves the clarity of the solution. This method compares favourably with standard Technicon stannous chloride method. ($r = 0.981$).

Introduction

The usefulness of the standard Technicon stannous chloride method is limited by the appreciably large volume of sample required (0.23 ml) and problems with minor deviation from linearity at high levels of phosphate. Also,

one of the reagents is made up in concentrated acid which introduces a definite hazard.

A number of direct methods have been reported in the literature^{1,2}. These have the apparent advantage of increased sensitivity and therefore decreased sample volume. The method of Bloore¹ was chosen for further study.

Materials and Methods

1. Standard SMA12/60 Technicon stannous chloride method (number XII). Reagents as specified in the SMA12/60 handbook³.

2. New Method. The equipment was a standard SMA12/60 cartridge with the arrangement as shown in Figure 1. The reagents were prepared as follows (all chemicals BDH, LR grade unless otherwise stated):
 (a) Reducing Reagent: Metol (p-methyl ammonium phenol sulphate), 1.50 g, Sodium Metabisulphite 4.50 g, Sodium dodecasulphate (especially pure) 10.0 g, distilled water to 1 litre.
 (b) Ammonium molybdate reagent: Amonium molybdate (4H₂O), 10.0 g, (May & Baker, LR), Sulphuric Acid concentrated (AR) 55.5 ml, distilled water to 1 litre.
 (c) Triethanolamine 200 ml, distilled water to 1 litre. All reagents were stable at least two months at room temperature.

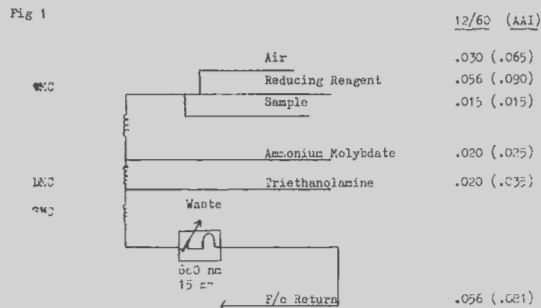


Fig 1
 All tubes are flow-rated and sizes given in inches (internal diameter).
 DMC = Double Mixing Coil.
 SMC = Single Mixing Coil.

Results

One hundred and eighteen samples were run to compare the standard stannous chloride method with this method. A regression analysis was performed on the two sets of results giving $r = 0.981$, $a = 0.021$, $b = 1.016$ where r = correlation coefficient, a = intercept and b = slope.

Precision was measured by 20 determinations of a sample with a level of 1.45 mmol/litre

giving a coefficient of variation of 0.3 percent. Blanks were run on a series of jaundiced and lipaemic sera by omitting the colour reagent (ammonium molybdate). No measurable peaks were obtained on the recorder. Washout and carryover characteristics are shown in Figure 2. Recovery tests for specificity were performed with 97-100 percent recovery.

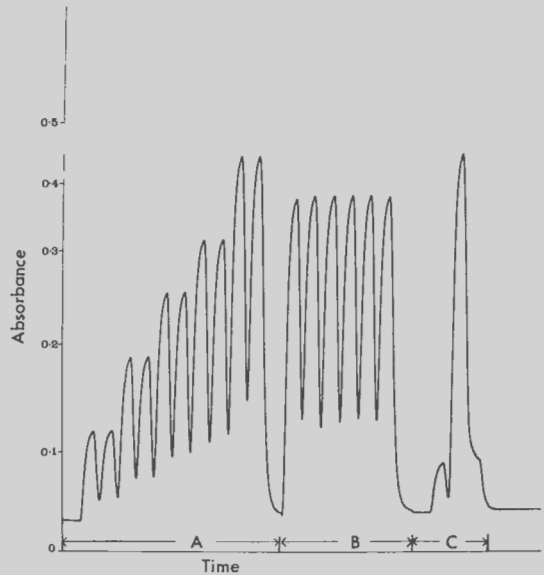


Figure 2. Recorder tracings of (A) duplicate phosphate standards of 0.5, 1.0, 1.5, 2.0, 3.0 mmol/litre, (B) replicate samples of 2.6 mmol/litre, (C) 0.25, 3.0, 0.25 mmol/litre samples showing interaction between high and low values.

Discussion

When the method of Bloore was initially adopted for use on a continuous flow system, trouble was experienced with precipitation of proteins. Various surfactants were tried to overcome the problem. Of those tried, Nonidet P40, Triton X-100, Brij 35, FC-95 and Sodium dodecasulphate, only sodium dodecasulphate at levels greater than 0.5 percent w/v were found to be successful. Due to the problems involved with solubility of the compound at levels greater than 2 percent w/v, a 1 percent w/v solution was incorporated with the reducing reagent and it also acted as the method wetting agent. This strength sodium dodecasulphate will keep all normal proteins in solution, but levels of abnormal gamma globulin above about 50 g/litre will precipitate. This phenomenon only occurs a few times a year and so it was not considered to be a great

disadvantage. When used with prediluted sample the abnormal proteins do not normally precipitate.

A sample rate of 60 per hour and sample/wash ratios of 2/1 for AAI systems and 9/1 for AAI systems were found to be suitable.

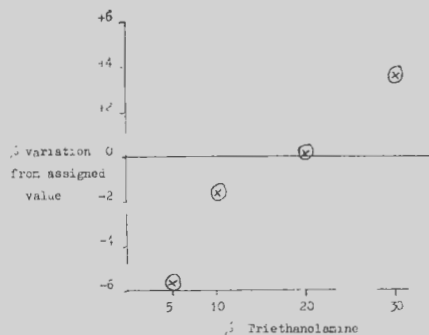
With the original method of Bloore, poor correlation was found between serum and aqueous standards. This was found to be due to the strength of the Triethanolamine. The original strength of 30 percent triethanolamine was suitable for a system which utilised a serum standard; but for AAI systems which use an aqueous standard curve the level of triethanolamine must be dropped to 20 percent v/v to give good correlation with the standard Technicon method. Levels of 5 percent and 10 percent v/v were found to be unsatisfactory due to baseline noise and poor correlation with the standard method, Figure 3.

This method as described, requires 0.07 ml sample for AAI system and 0.1 ml of prediluted sample for AAI systems, and is therefore a suitable micro method for both analyser systems.

Conclusion

A quick, simple and relatively inexpensive method for the direct measurement of inorganic phosphate is presented. The only problem arises with high levels of gamma globulin which may precipitate with the ammonium molybdate. A suitable dilution of the serum will overcome the problem.

Fig 3



Plot of percentage variation from the given value of selected sera using various strengths of Triethanolamine and reading the peaks with a curve drawn using aqueous standards.

Figure 3. Plot of percentage variation from the given value of selected sera using various strengths of Triethanolamine and reading the peaks with a curve drawn using aqueous standards.

Acknowledgments

My thanks to J. Powell for guidance and assistance and to Dr C. Small for the composition of the article.

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Abstracts of Theses presented as a Requirement for Partial Qualification as a Qualified Technical Officer 1975

Evaluation of Faecal Leucocytes in Relation to Intestinal Disorders.

Iosefa Tesese, Auckland Hospital, January, 1975.

The presence of leucocytes in faeces from patients with various gastrointestinal pathologies is of interest as a form of diagnostic tool. This article gave adequate detail of techniques

investigated with comment on each. Routine microbiological identification procedures were employed to identify organisms isolated from this study of 1,436 faecal specimens and documentation was made of nature of specimen, presence of leucocytes and leucocyte type. A correlation was attempted with some success between these findings and the organisms isolated.

A Comparison of Staining Techniques in the Histological Identification of Amyloid in Human Tissues.

I. Maria Pap, Green Lane Hospital, Auckland. March, 1975.

No one procedure can be regarded as absolutely amyloid specific, although some procedures are more useful than others. Most satisfactory results came from the combined use of several stains on any one tissue.

In this study the Haematoxylin and Eosin stain was used as a guide for initial recognition of amyloid. The findings of homogeneous eosinophilic areas were regarded as likely to be amyloid but not specific. Some workers seem to be quite satisfied in diagnosing amyloid by the finding of an amorphous acidophilic material in Haematoxylin and Eosin stained sections.

Sirius Red stain showed positive results on all sections and the staining on them seems to keep well, therefore it would be recommended to use as one stain for routine diagnosis.

The Methyl Violet stain also gave positive results in this study, but showed bleeding and fading which could only be partially overcome by using modified mounting media.

The Congo Red stain gave positive red staining in this study, but difficulty was encountered with the examination of this stain by polarised light.

The van Gieson's stain for amyloid gave positive results in this study, but the colour was variable and fading was detected after 12 months.

The sodium sulphate Alcian Blue method also gave positive results and is able to show the most details of amyloid deposits. The value lies in the subsequent counterstaining which reveals the structural relationships of amyloid. In spite of its long staining time and the necessity for meticulous care with the method it may also justify adoption as an alternative method for routine diagnosis for amyloidosis.

One of the aims of the survey was to determine the effects of prolonged fixation and storage on the staining properties of amyloid. The observations and careful examinations showed that they have no marked effect on staining within the period of study. No formalin pigment formed in any of the sections.

Amyloid in processed, wax embedded, states retains its ability to stain brightly over long periods.

Amyloid in cut paraffin sections which have been attached to slides can be stored as it does not lose its staining power for at least one year.

Thick section technique (12 microns) has its advantage in demonstrating minute amyloid deposits, but should be used in parallel with five microns sections which show cytological detail.

A Comparison of BAGG Broth and Bile-aesculin Medium for the Presumptive Identification of Group D Streptococci from Urines.

Josephine M. Tetlow, Medical Laboratory, Auckland. March, 1975.

The object of this investigation was to compare the accuracy of BAGG broth and bile-aesculin medium for the identification of Group D streptococci under routine working conditions.

Streptococci isolates collected from urines over a three-month period were inoculated into conventionally prepared bile-aesculin agar, bile-aesculin broth and BAGG broth and also into batches of media which were refrigerated and heated excessively, the results being read at 24, 48, and 72h incubation.

Identical results were obtained in freshly prepared media and media stored at 4°C for four weeks. Overheating appeared to have little effect. No more positive results were obtained on further incubation of the bile-aesculin media; in fact on prolonged incubation more false positives were obtained. However BAGG broth incubated an extra 24 hours increased the number of positive results by 3 percent.

It was therefore concluded that the media in question all gave comparable results sufficiently accurate for routine use in identifying Group D strains of streptococci from urines even when small inocula were used and were incubated for only 24 hours at 37°C.

Comparison of an Enzyme Extraction Method of C-Carbohydrate of B-Haemolytic Streptococci Using Pronase B, with the Standard Rantz and Randall Method of Extraction and the Lancefield Grouping by (1) Precipitation Method, (2) Gel Diffusion, (3) Counter Immunoelectrophoresis.

Tamara Schulze, Microbiology Department, Christchurch Hospital, Christchurch. April, 1975.

A full description of methods and a list of materials is given. A total of 100 Beta haemolytic streptococci were examined by each method. Gel diffusion using the Rantz and Randall extraction was used as a standard for comparison. Ninety-six percent of Rantz and Randall extraction grouped by capillary tube compared with 24 percent by Pronase extraction and 86 percent of Rantz and Randall grouped by counter immunoelectrophoresis compared with 42 percent of Pronase B extraction method. Using the Pronase B and capillary tube method almost half precipitated non-specifically.

A breakdown of all groups of Beta haemolytic streptococci tested showed that the Rantz and Randall-gel diffusion was a satisfactory combination. The percent of grouping with Pronase B extraction was very low in all groups tested. Using a capillary precipitation method the Rantz and Randall extraction was far superior to the Pronase B method.

Overall the counter immunoelectrophoresis method proved disappointing.

A Survey of Urinary Cytology.

Mrs Rachel F. Carnielo, Cytology Laboratory, National Women's Hospital, Auckland. April, 1975.

The thesis describes a cytological survey for urinary cancer carried out in Auckland. A total of 610 random urines from 533 patients, mainly from the Auckland Hospital and Green Lane Hospital clinics and wards, were examined. Cytological findings were graded from one (absence of atypical or abnormal cells) to five (cells conclusive for malignancy).

Centrifuged sediments were smeared on slides then sprayed with "Cytofix", mainly to provide good adherence of cells to the slide, followed by fixation in 100 percent alcohol. Slides were stained by Papanicolaou's method. It was found that one malignant sediment slide was capable of contaminating all slides in the same Coplin jar.

Drugs, catheterisation and inflammatory conditions caused cellular atypias which could give rise to diagnostic errors. Five specimens contained malignant transitional cells, four of these cases were already confirmed histologically, the fifth was later confirmed. Urines from four patients with carcinoma of the prostate did not yield positive results in cytology. Three tables show (1) urinary cytology results allied with symptoms, (2) microscopic finding of

haematuria and (3) Broders Grading Classification of Bladder-tumours. Fifteen plates show various casts, crystals and cell types found in the urine specimens.

The conclusion indicates that "a mass screening programme for the detection of occult bladder neoplasms would be of doubtful value in asymptomatic patients". The survey shows cytology to be of some value in patients with certain urinary complaints and as a useful diagnostic tool in clinically suspected cases of bladder neoplasm.

A Modified Latex Method for the Estimation of Fibrin(ogen) Degradation Products and the Significance of Results in Various Clinical Situations.

Leonie Elizabeth Caulfield, Auckland Hospital. April, 1975.

Tests for fibrin degradation products (FDPs) have become yet another clinico-pathological tool relatively simple to perform and of most useful clinical importance. This article looks briefly at the normal mechanism of fibrinolysis and summarises concisely the characteristics of FDPs. The technical method employed, that of a latex particle technique based on "Thrombo-Wellcotest" includes a modification which gives a greater range of dilution of test sera than that stated in the maker's data sheet, thus giving a better serial indicator of patient progress. Results of studies of various clinical states are documented and discussed. Eleven references are attached, some of which are excellent reading on the subject of FDPs.

A Spectrophotometric Analysis of Factors Affecting the Staining Properties of Haematoxylin Solution.

Miss Ien Oey, National Women's Hospital, Auckland. April, 1975.

Two hundred and twenty separate spectrophotometric analyses, of simple haematoxylin solutions, were carried out in an attempt to clarify some of the factors that determine the final staining ability of haematoxylin stains. The conclusions reached were:

- (1) The staining component of haematoxylin solution shows an absorption peak at 560 μ .
- (2) The fully over-oxidised haematein complex shows an absorption peak at 370-390 μ .
- (3) There is a minimal amount of mordant required to give stable optimal absorption peaks. Excess mordant does not appear to affect the spectrophotometric curves. The addition of mordant broadens the haematein peak at 560 μ .

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- (4) Spectrographic examination of new batches of haematoxylin is best carried out at pH 8.0 when determining the amount of naturally occurring haematein present.
- (5) Preliminary evidence suggests that much smaller amounts of iodate than customary may be used.
- (6) Absorption peaks display pH dependence.
- (7) Glycol as well as being a good solvent for haematoxylin appears to play a genuine role in retarding artificial chemical oxidation.

A Short Investigation into the Fibrinolytic Activity of Coronary Care Patients.

Yvonne J. Pannett, Kew Hospital, Southland. 1975.

It has been suggested that patients with a recent history of myocardial infarction have decreased fibrinolytic activity. Studies were undertaken using the Hyland Enzo-Diffusion Fibrin Plate Test with emphasis on available plasmin, active plasmin and total plasminogen. Details of technique are documented and the results obtained of the 80 cases studied. However, normal values were not well defined and although no firm conclusions were reached, several points of interest in this field were raised in discussion.

Book Reviews

Basic Histology. L. C. Jungueira, J. Carneiro and A. N. Contopoulos. Published by Lange Medical Publications. 440 pages; \$NZ12.65. Supplied by N. M. Peryer Limited, Christchurch.

This text is a further edition to the range of medical publications by Lange Medical Publications, and is the first edition in English of a text utilised in Brazilian Medical Schools.

The outlay of this book is similar to most Lange publications in that the page layout is double column and the numerous subheadings within each paragraph conveniently underline each important section of the text. Numerous schematic diagrams, photomicrographs and electronmicrographs, each with an accurate description, supplement each section of the text.

The initial three chapters are allocated to histological methods of study, histochemistry and cytochemistry. Thereafter each chapter is allocated to a specific tissue group within the human body, and includes an introduction which covers the relevant basic physiology and anatomy. Throughout no attempt is made to search each section in depth. Rather the fundamental aspects are covered well and relevant, accurate, and up-to-date references are supplied for further reading.

There can be no doubt that this book fulfils its promise of a text of basic histology by covering each body tissue accurately without prejudice to another. As with most Lange publications it will certainly find itself acquired by many hospital personnel and will serve a useful function in their hands.

Bert White.

Immunology and Serology, third edition, 1975.

Philip L. Carpenter. Published by W. B. Saunders and obtained from N. M. Peryer Ltd., Christchurch. 346 pages, illustrated. Price \$NZ14.25.

Ten years have elapsed since the previous edition and during this time there has been a considerable advance in the subject. The structure of the immunoglobulin molecule has been defined. There has been some elucidation of humoral and cell mediated immunity and the B and T types of lymphocytes. We now know that complement covers a multitude of components. All of these advances are included in this edition.

The book is planned to provide an historical and physiological introduction which covers the composition of the blood, the immunologically active tissues, humoral and cellular immunity, the proteins and immunoglobulins. The bulk of the book covers various aspects of antigen-antibody reactions and concludes with a practical appendix.

Random points of interest include reference to adoptive immunity by the transfer of antigen-sensitive lymphocytes to a non-immune recipient. The expression "immunogen" is used to differentiate agents that illicit an immune response, restricting "antigen" to substances that react with lymphoid cells or cell products. The immunoglobulins are discussed in great detail but although there is reference to various abnormal conditions throughout the book gammopathies are evidently not considered to come within its

scope. The chapters on "Biologic aspects of immune reactions" and "Antigen-antibody reactions" are concluded with summaries. I wish the other chapters had been concluded in this manner because due to the terse textbook style and unfamiliar terminology I found it heavy going! Conglutination did not receive a mention.

Immunology requires a wide range of laboratory tests and these are described in theory and in practice. Flocculation test for syphilis, C reactive protein, forensic precipitation tests, microbiological serology, complement fixation and blood cell grouping are all dealt with. The complement cascade is thoroughly discussed in relation to complement mediated cytotoxicity. Virus immunology, toxin neutralisation, hypersensitivity, autoimmune disease, tissue transplantation and tumour immunology are also discussed. (The two latter topics, briefly.)

The final chapter entitled "Experiments in Serology," contains succinct accounts of technical procedures including blood counts and grouping, complement fixation, fluorescent antibody techniques, phagocytosis, preparation of immune material and the inoculation and bleeding of animals.

This book should be of interest to those considering specialisation in immunology. It was intended as a graduate student text assuming some knowledge of basic science subjects and might well cover the theoretical requirements for a laboratory examination in the subject. Further details on technical matters would have to be sought elsewhere.

R. D. Allan.

Primer of Serology, first edition, 1975. Adrian N. C. Delaat, ART. Published by Harper and Rowe, Hagerstown, Maryland, USA. 148 pages, 56 illustrations, size 6in by 9in. Paperback with index, Price \$US9.95.

It is a coincidence that two books on the same topic and covering the same subject matter arrived for review at the same time and inevitable that one should compare them.

This little book is primarily intended for students in medical laboratory technology, the topics are treated rather simply and it consists mainly of descriptions of the various techniques in serology. The previous book which is intended for academic instruction is more than twice the size and discusses the subject at greater depth. This primer of sero-

logy possibly fulfils a need in the American schools of medical technology.

The initial chapter on basic principles describes and defines terms used in immunology and complex antigens are graphically depicted in a series of ingenious diagrams. These diagrams are a feature of the book and are used throughout to illustrate antigen-antibody reactions. Precipitation covers the various forms of gel precipitation including diffusion test and immunoelectrophoresis the VDRL and RPR test. It includes some familiar illustrations, some from kitsets, and the AA manifold diagram for the reagin test.

Agglutination relates to microbiological tests and mentions Latex fixations tests with respect to rheumatoid factor. The various types of haemagglutination are diagrammatically illustrated, blood grouping glanced at as are tests using the principle of haemagglutination. Complement was not satisfactorily dealt with to my mind. There is little point in mentioning the various components without explaining their function. The technique of complement fixation was explained.

Neutralisation, immunofluorescence, radio-immunoassay were also similarly treated. The last chapter consists of miscellaneous techniques.

The introduction refers to serology as a hybrid science and its presentation as a variety of techniques certainly emphasises its chimerical character. The book might serve as an introduction but for satisfactory understanding use would have to be made of the liberal references concluding each chapter.

R. D. Allan.

Opportunistic Fungal Infections. Proceedings of the Second International Conference. Edited by Ernest W. Chick, Albert Balows, Michael L. Furcolow (American Lecture Series in Clinical Microbiology). Charles C. Thomas, Springfield, Illinois, USA. 1975, 359 pages. Price \$US22.50.

The Second International Conference on Opportunistic Fungal Infections was held in March 1972 at Lexington, USA, 10 years after the first such conference. Many notable international authorities participated and the 28 papers presented during the two days are published in this volume with a preface and a foreword by two of the editors. The dust-cover, binding, paper and printing are all of

impeccable quality. To handle and read the book is a continuous pleasure. Only seven of the papers are accompanied by black and white plates and these also are of the highest quality. It is to be regretted that, unlike other similar publications, this one does not provide the professional qualifications and addresses of the authors. Only for the three editors is such information given. Errors in spelling and variations in the names of both authors and fungi cannot be overlooked and in a lengthy table for one paper they are embarrassingly abundant.

The reader is left with a feeling of uncertainty and the need to verify every unfamiliar word. In view of the three-year lapse between the conference and the publication, it is difficult to understand how so much could have escaped the proof readers. The designation, "opportunistic fungus", has been the subject of controversy and the contributors discuss the continuing and unresolved problem of defining an opportunistic fungal infection. Since no acceptable alternative has been put forward the words are now used to describe infections which may be summarised as resulting from a predisposing condition or from the use of a therapeutic agent.

The causative fungi may be endogenous, as with *Candida albicans* or *Torulopsis glabrata*, or exogenous as with *Cryptococcus neoformans* or *Aspergillus fumigatus*.

The 28 papers are grouped in sections under six headings:

1. Scope of opportunistic fungal infections.
2. Serological and immunological aspects.
3. Clinical aspects.
4. Specific diseases.
5. Veterinary aspects.
6. Therapeutic aspects.

All four papers in part one are outstanding as would be expected from authors such as H. P. R. Seeliger, C. W. Emmons and L. Ajello.

The comparatively brief paper by Ajello "Problems in the Laboratory Diagnosis of Opportunistic Fungal Infections," should be read by all microbiologists in New Zealand and recommended to each trainee in this discipline. Only the copyright injunction in front of the book restrains the suggestion that copies be placed on the walls of every department of microbiology.

The five papers in the serology section (and

one from the general section is really concerned with this topic) are valuable for an understanding of the difficulties of this aspect. The situation at the time of the conference is well summarised by F. E. Tosh in stating that "serious problems still exist in the serological diagnosis of most, if not all, of the fungal diseases." The two papers by L. Kaufman and W. Kaplan, both of the Center for Disease Control, Atlanta, indicate how important serological methods may be in the diagnosis of infections caused by opportunistic fungi.

The most interesting paper in the third section is that of D. B. Jones on "Fungal Keratitis." It is particularly useful since fungal infections of the eye are not common in New Zealand. The graphic series of photographs of infected eyes and of the method for scraping a corneal ulcer adds to the value of this paper.

Throughout the book we are confronted with the varying terminology for infections due to yeast-like fungi of the genus *Candida*. Undoubtedly "candidiasis" will continue to be used, although the plea for "candidosis" has been made for 12 years by English and European authorities. In a paper by M. L. Dillon on "Opportunistic Fungus Infections in Surgery" a new use of "candidiasis" is found with references to "candidiasis cultured from the venous blood" and "candidiasis isolated from the tip of a catheter".

The 10 papers in the section on specific diseases are of importance commensurate with the status of the author. Thus the papers of H. I. Winner on "Candidosis", N. F. Conant on "Nocardiosis" and R. D. Baker on "Mucormycosis" are the classical review articles that would be expected. Professor W. St C. Symmers' paper on "Histopathology of Opportunistic Fungal Infections" is a long and scholarly one with numerous illustrations. It makes engrossing reading for microbiologists who may not be familiar with histopathology. Included in this fourth section is a paper by E. W. Chick on "Opportunistic Fungi as Producers of Mycotoxins". The paper consists of two pages, followed by a table of 13 pages with 10 pages of references. While mycotoxicology is a field of tremendous importance, its place in this conference is debatable. Overlooking this aspect, the chief point of criticism is that the table, citing the names of numerous species of fungi, contains so many errors in both specific and generic names. For an ex-

perienced mycologist, detecting the mistakes may be a stimulating exercise, for the uninitiated it can only be a misleading confusion.

With one exception, the papers in the veterinary and therapeutic sections are not of the same standard as the preceding sections. The exception is "The Medical Treatment of Patients with Opportunistic Fungal Infections" by J. P. Utz. Dr Utz's textbook on Medical Mycology is now well known in New Zealand and this paper evaluates meticulously the drugs available as "established first choices or as promising new agents". This will be a useful reference for the microbiologist who, on reporting such a fungus, is confronted with the question, "What is it sensitive to?"

The very great value of the book as a whole is only emphasised by the imperfections noted by this reviewer. It should be in the library of all hospitals and medical laboratories in New Zealand and it should be read and studied by all microbiologists. As Dr Ajello states in the last sentence of his paper, "Only the prepared mind can aid the impaired host".

F. M. Rush-Munro.

Clinical Bacteriology. E. Joan Stokes. Published by Edward Arnold Ltd. Fourth Edition, 1975. Price \$NZ19.90. 394 pages, illustrated. Available from Book Representatives (New Zealand) Ltd., Northcote, Auckland.

The fourth edition of this book appears 21 years after the appearance of the first edition in a soft cover form enlarged from the first edition by some 50 pages. This book still retains its usefulness as a basic bench book for the laboratory assistant, technologist, and pathologist. Most people could improve their bench technique by following the author's scrupulous and well-proven methods.

Chapter two sees the introduction of a section on computer aids in microbiology records and reporting, and brief mention on quality control which could perhaps have been allotted greater space and more detail. The section on blood cultures has been revised in line with current techniques, whilst the section on the identification of bacteria shows little change, apart from the adoption of the nomenclature as used in the sixth edition of Topley and Wilson. The section on anti-bacterial drugs has received the greatest attention; whilst still advocating the controlled paper disc diffusion method, no mention has been

made of the Kirby Bauer method. Methicillin resistance is very briefly discussed.

The author commits to print her views on the cross resistance of staphylococci with methicillin and cephalosporins, and advocates the testing of only the former, whilst reporting both antibiotics. Co-trimoxazole also gains recognition by the fact that only the individual components should be tested. The Immunology section has been revitalised by the alterations, such as the Vi agglutination test, the description of complement fixation tests in general, and the inclusion of the TPHA test. For a book of this nature the media section could well be expanded even though there are new additions to the list and obsolete ones have been deleted. Anaerobic organisms and techniques have been rather skimmed over, considering their importance.

In spite of the book's deficiencies, it is still a useful and helpful bench guide to microbiologists in general, and in particular to the trainee. Although virology is omitted, the inclusion of sections on hospital infection, bench techniques and the valuable introductory chapter on the philosophy of clinical bacteriology make the book a worthwhile addition and reference book for all laboratory personnel providing one does not expect a specialised reference manual.

D. A. Robertson.

Microbiology—1975. Edited by David Schlesinger. Published by the American Society for Microbiology, Washington, D.C., U.S.A. 514 pages. Price \$US16.00.

Microbiology—1975 is the second in this series. This book is divided into five sections and as a reviewer I feel I cannot adequately comment on two of the sections: those on Mycotoxins and on Cell Differentiation and Communication.

However the other three sections contain much material of interest to both technologists and the clinical microbiologist. The first section on Rapid Diagnostic Techniques in Clinical Microbiology contains papers on impedance measurement techniques, microcalorimetry, bio-luminescence, radiometric detection of bacterial growth, radioenzymatic antibiotic assay method, gas-liquid chromatography for identification of organisms and diagnosis of septicaemia and meningitis, immuno-electrophoretic methods for the detection,

identification and quantitation of bacterial infections, finally two papers on computer data storage and the application of computers to hospital epidemiology.

Many of the papers in this section will be of interest but the application of these newer techniques need to be evaluated in the routine diagnostic laboratory. The immuno-electrophoretic methods appear to be the ones which could most easily fit into the present day microbiology laboratory.

The use of gas-liquid chromatography particularly in the rapid diagnosis of meningitis, septicaemia and other diseases is currently being evaluated in a number of centres. GLC linked with other techniques using pyrolysis and mass spectroscopy may in the future change the whole role of present day methods in hospital laboratories processing large numbers of specimens and cultures.

The second section on Pathogenic Mechanisms in Bacterial Diseases is again divided into sub-sections of interaction at body surfaces, penetration, inflammation and cellular events, exotoxins and pathogenic mechanisms in opportunistic gram-negative bacillary infections.

Each of these sub-sections indicates the present interest in pathogenic mechanisms and so in turn allows the technologist to understand the basis of infectious diseases and the clinical microbiologist to direct the most suitable form of treatment.

The section on new vaccines discusses vaccines and immunity with *Haemophilus influenzae*, *Neisseria* (both meningococcus and gonococcus), respiratory virus vaccines, and the control of the *Pseudomonas aeruginosa* infections. Each of these sub-sections deal with diseases which are of sufficient importance to ensure the continuance of research and development on these specific vaccine and immunoglobulins. Overall this volume has much to recommend it to all those associated with medical microbiology at both the technical and clinical level. If future volumes are as widespread in their content as this one we can look forward to a worthwhile series.

G. R. Rose.

Bacteriological Code of Nomenclature of Bacteria. Edited by S. P. Lapage, P. H. A. Sneath, E. F. Lessel, V. B. D. Skerman, H. P. R. Seeliger and W. A. Clark; American Society for Microbiology, Wash-

ington, D.C., December 1975, 108 pages, Price \$US5.00.

This hard covered text contains a five-page memorial to Professor R. E. Buchanan, an American bacteriologist, famous world wide as chairman of the Bergey's Manual Trust and joint editor of Index Bergeyona.

Thirty pages of prefaces, forewords, and supplements to forewords are also found at the front of the book and these help to explain the aims and objectives of this current edition.

The remainder of the book deals with bacterial taxonomy in all its nuances and the International rules which have been set up to ensure that new organisms are properly classified and recorded.

Useful suggestions are made regarding the storage of a new organism and ensuring that the minimum number of subcultures are made when maintaining the strain for posterity.

This book should be available from the university or hospital library but is of only limited interest or use in the average hospital diagnostic microbiology laboratory which of necessity is concentrating on the rapid diagnosis and antibiotic sensitivities of bacterial isolates. The unusual or atypical bacterial isolate is more likely to be forwarded "elsewhere" for detailed taxonomy and classification and this book should be a valuable text for reference laboratories dealing with these unusual or atypical organisms.

Joan L. Faoagali.

Medical Microbiology, Volume Two, Twelfth Edition. 587 pages, illustrated. R. Cruickshank, J. P. Duguiel, B. P. Marmion, R. H. A. Swain. Published Churchill Livingstone and obtained from Penguin Books (N.Z.) Ltd., Auckland. Price \$NS20.70.

Medical Microbiology, Volume Two, is intended as a "bench book" for professional and technical staffs of medical, scientific and veterinary laboratories. The authors comment in the preface that each chapter has been revised, that some have been rewritten and several new chapters have been added.

The volume is divided into two sections: Part 1 which covers 346 pages is devoted to technical methods, while the 212 pages in Part 2 relate to the identification of microorganisms and the laboratory diagnosis of specific infections. The book contains, with some additions, the technical data from the eleventh edition.

The first 109 pages which include microscopy, staining methods, sterilisation, pH measurements and the cultivation of bacteria and fungi have been uplifted virtually unaltered from the previous edition.

The section relating to culture media has been enlarged with the addition of 26 new formulae. These additions include a section devoted to media for the cultivation of mycoplasma.

Some additions have been made to the chapter dealing with sensitivity testing to antimicrobial agents. These include a list of neutralisers which may be used when testing the efficiency of disinfectants and some additional tests including the in-use test.

The chapter devoted to immunological and serological methods has been re-arranged and is well illustrated.

A very welcome addition is a chapter dealing with safety in the microbiology laboratory. The occurrence of laboratory infections, routes of infection, safety organisation and the use of pathogens in teaching are discussed in this chapter.

Part 2 of the volume commences with some brief comments regarding the function of staff, reporting of results, epidemiological investigations and training of microbiologists. The rest of Part 2 is devoted to descriptions of various micro-organisms and their laboratory diagnosis.

Medical Microbiology is an authoritative textbook written in the same lucid style which has been the hallmark of its long line of predecessors. For all its excellent qualities however, it is a "bread and butter" textbook. One would have hoped that with the evolution of Medical Microbiology into two volumes and an increase in price to over \$40 the text, particularly in Volume Two, would have been expanded to meet more adequately the current needs of both the microbiology laboratory and the senior student.

The common bacteria are dealt with in considerable depth but this book is little help with the less common organisms. They receive very superficial treatment, or are completely ignored. The very superficial treatment of *Listeria monocytogenes* may be taken as an example. The absence of a method for the rapid assay of serum aminoglycoside levels is surprising. One also wonders why tests relating to the quantitation of streptococcal

antibodies such as the antistreptolysin O test continues to be omitted.

In spite of these limitations Medical Microbiology is still a useful textbook to have on the laboratory bookshelf. It must also be recognised as one of the better textbooks for the student technologist, particularly up to the Basic Training Certificate level. Beyond this point however, the student would be wise to read more widely.

A. F. Harper.

Practical Haematology. J. V. Dacie, S. M. Lewis. Fifth Edition 1975. 629 pages, illustrated, Published by Churchill Livingstone and obtained from Penguin Books (N.Z.) Ltd, Auckland. Price \$NZ17.35.

It is always pleasant to receive a new edition of a good textbook and Practical Haematology is no exception. The authors have found it necessary to increase its size by some 60 pages of new material in spite of extensive revision and deletion of obsolete methods. In common with other modern textbooks they have enlisted the support of a number of collaborators some of whom have been responsible for the preparation of specific chapters. Whilst this produces differences in style of presentation it does bring together a pooling of expert knowledge which enhances the standing of the book.

The authors have rearranged some sections notably that on haemolytic anaemias which now encompasses five chapters, grouping all the laboratory methods in systematic order. There is a new chapter on leucocyte and platelet antigens and antibodies and that on blood coagulation tests show extensive revision and includes up to date methods. SI units are used throughout which is most useful considering their recent introduction into New Zealand. In the chapter on red cell abnormalities the number of very excellent photomicrographs has been increased to forty-nine and bear the unmistakable descriptions of Professor Dacie himself.

Technologists in haematology should dispose of their old editions and obtain the new without delay.

B. W. Main.

Cell and Tissue Culture. Paul J. Churchill. Livingstone. Illustrated, also colour plates. 1975, Fifth edition. 484 pages, Price \$NZ 21.70. Obtained from N. M. Peryer Ltd, Christchurch.

The name of this author will be familiar to those involved in cell culture and related work through the earlier editions of this volume. The author has frequently revised the book since it first appeared in 1959 and it has now reached the fifth edition.

The author sets out initially to provide a "how-to-do-it book" on techniques and their application in the field of cell and tissue culture. The new edition follows the same pattern, but it has been revised in many areas. In this regard, the author whose experience spans the growth and development of modern cell and tissue culture has still retained a significant number of the older simple culture techniques and these provide an interesting historical perspective as well as demonstrating that simple equipment can be used for some procedures.

The book is divided into four sections. The first deals with the principles of cell physiology and biochemistry, and the basic requirements and behaviour of cells in culture. This serves as a very useful introduction to the novice in the cell culture field. The second part deals with the preparation of materials used in tissue culture and is a sound practical review of the problems of contamination, cleaning procedures, sterilisation and aseptic technique. This is again mainly of value to the beginner, although for those plagued with episodic problems with infections or other aspects of quality control in cultures, a re-reading would be of value. This section however provides surprisingly little discussion of the place of laminar flow cabinets in cell culture laboratories; such cabinets are major items and a comprehensive review of their actual value would be timely.

The third part deals with specific techniques of manipulating cells and tissues and propagation for different purposes, and also includes chapters on culture of cells from cold-blooded animals and plants. This and the following section on applications of cell culture techniques has undergone revision in some areas with some useful additions. There are also entirely new chapters on applications in genetics and cancer research.

This is essentially a practical book on methodology which attempts to serve as an introduction to the subject of cell and tissue culture. It will serve as a useful introduction to the novice on cell and tissue culture and will also provide a useful introduction to those moving

into new fields of culture work where they have little experience. However the field covered by the book is now sufficiently complex that no single volume of this size can now do more than provide a superficial coverage and this is clearly apparent in such chapters as that on applications in cancer research. This is also apparent in the references where the author supplies references for the methods quoted and also to some other selected authors but the references are by no means comprehensive and few references later than 1971 are present. Despite this I consider the book will be of considerable value like its predecessors and will probably achieve the same high popularity.

J. M. Faed.

The Color Atlas of Intestinal Parasites. Francis M. Spencer, AB, MD, FACP, and Lee S. Monroe, MS, MD, FACP. 1975, Sixth Edition. 158 pages. Published by Charles C. Thomas, Springfield, Illinois, U.S.A., Price \$US14.75.

Two hundred and forty-eight reproduced colour photomicrographs show the intestinal parasites exactly as they appear through an ordinary microscope. Because these organisms are often in different stages of development and many vary in shape, size and structure making identification difficult a large number of photomicrographs have been included to show this variety. For example there are 12 photomicrographs alone of the *E. histolytica* trophozoite showing different forms, using both trichrome and iron haematoxylin stains. In most cases the organisms are shown as they appear in saline preparations as well as in commonly employed stains.

The greater part of the text is supplementary to the colour atlas giving clear descriptions of what one sees microscopically. As it is not intended as a textbook of parasitology but as a technical aid, one would have to look elsewhere for details of life cycles and epidemiology. However brief mention is made of distribution and pathogenicity of each species.

Eighteen pages are devoted to laboratory methods including collection of specimens, concentration, fixing and staining methods and notes on the serological diagnosis of intestinal parasitosis.

A very valuable chapter on confusing objects found in faeces supports many photomicrographs of pseudoparasites and harmless com-

mensals which are often mistaken for important parasites.

This book would be an asset to any clinical laboratory but especially so in New Zealand where the number of parasites seen is not very great and consequently the laboratory technologist has little practice in recognising the less common species.

Maree Johnstone.

Books Received

The Laboratory in Clinical Medicine. Interpretation and Application. James A. Halsted, M.D. 1976, First Edition. Published by W. B. Saunders Co. and obtained from N. M. Peryer Ltd., Christchurch. 866 pages.

illustrated. Price \$NZ35.75.

This book is the successor to four editions of Clinical Pathology first written in 1950 by Dr Benjamin B. Wells. It contains a wealth of up-to-date information.

The Lab Aide. Leslie Lee, B.S., M.T. (ASCP). Published by C. V. Moseby Company, St Louis, and obtained from N. M. Peryer Ltd., Christchurch. 140 pages, illustrated, Price \$NZ6.85.

Economy has dictated that non-technical personnel must be used for many jobs in the hospital laboratory and this book has been written to help them to become familiar with the laboratory and its functions.

Abstracts

Contributors: D. G. Bolitho, J. Hannan, N. Langford, L. M. Milligan and Janice Parker.

Clinical Biochemistry

Quantitative Fractionation of Alkaline Phosphatase Isoenzymes According to their Thermostability. Claude, Petit-Clerc, (1976), *Clin. Chem.* 22, 42.

A continuous flow system permitting simultaneous identification and quantitation of the placental, intestinal and liver isoenzymes of Alkaline Phosphatase. The method does not differentiate the isoenzyme of bone origin from other very heat labile forms. —J.P.

Evaluation of a Reagent Strip Method for Glucose in Whole Blood as Compared with a Hexokinase Method. Stewart, T. C. (1976), *Clin. Chem.* 22, 74.

The author compared the new updated 'Dextrostix' (Ames Co.) with a potential national standard method for measurement of glucose in whole blood. Excellent correlation was obtained although operator variables were critical with the 'Dextrostix'. —J.P.

Sample Stability: A Suggested Definition and Method of Determination. Thiers, R. E., Wu, G. T., Reed, A. H. and Oliver, L. K. (1976), *Clin. Chem.* 22, 176.

A method is given for determining the stability of any chemical constituent of stored samples. From the data obtained a stated period and exactly defined conditions for storage can be proposed. —J.P.

Assessment of a Serum Separator Device for Obtaining Serum Specimens Suitable for Clinical Analyses. Laessig, R. H., Westgard, J. O., Carey, R. N., Hasseiner, D. J., Schwartz, T. H. and Feldbruegge, D. H. (1976), *Clin. Chem.* 22, 235.

Vacutainer tubes containing a semi-solid silicon polymer were used in parallel with ordinary vacutainer tubes and the results of a wide variety of assays compared. The only clinically significant change occurred in serum L.D.H. which increased 5-8 percent in the normal range. —J.P.

Introduction of a Vickers M300 Analyser into the Routine Service of a Hospital Laboratory. Buckley-Sharp, M. D., Miller, A. L., Stevens, J. E., Worsley, L. R., and Yeomans, F. R. A. (1976), *J. clin. Path.* 29, 322.

The Vickers M300 is a discrete analysis system designed to carry out up to 20 different tests at a rate of 300 samples/hour. The two papers relate to the installation of a Vickers M300 in a busy hospital laboratory. Discussion includes installation, staffing, logistics, tests, methods and a final evaluation of data. —J.P.

The Use of Enzyme Analysis of Peritoneal Blood in the Clinical Assessment of Abdominal Organ Injury. Delaney, H. M., Moss, C. M. and Carnevale, N. (1976), *Surg. Gynec. Obstet.* 142, 161.

Seventy patients with abdominal trauma and haemoperitoneum were evaluated by analysis of the enzyme content of peritoneal blood, using automated methods. The enzyme levels in peritoneal blood were evaluated relative to the simultaneous levels in peripheral blood to identify enzyme differences in organ-related peritoneal and peripheral blood samples. In patients with trauma to the small intestine, three of the four enzymes studied (Alk phos, LDH, GOT, CPK) had elevations of the mean values in the peritoneal blood relative to the peripheral blood. The most consistent enzyme elevation related to organ injury was the peritoneal alkaline phosphatase level in patients with injuries to the small intestine. —J.H.

The Effect of Multiphasic Biochemical Screening on the Diagnosis of Primary Hyperparathyroidism. Aitken, R. E., Bartley, P. C., Bryant, S. J. and Lloyd, H. M. (1975), *Aust. N.Z. J. Med.* 5, 224.

The main factor contributing to an increase from 2.5 to 11.7 cases per annum in the number of cases

of primary hyperparathyroidism diagnosed was the inclusion of plasma calcium determinations in the multiphasic screening programme, together with correction of the plasma calcium for the total protein concentration of the same blood sample. The normal range by the SMA 12/60 method using cresolphthalein complexone was established as 8.3 to 10.3 mg/dl. The corrected calcium value was obtained by dividing the total protein concentration by 14.4, adding 0.5 to this result, then dividing the observed calcium value by the sum. In a series of 3500 plasma calcium determinations, use of the correction equation excluded 21 results elevated because of raised total protein and revealed hypercalcaemia not recognised otherwise in 11 blood samples. —J.H.

Haematology and Immunohaematology

Classification of the Acute Leukaemias. Galton, D. A. G. and Dacie, J. V. (1975). *Blood Cells* 1 (1), 17.

A working numerical notation has been devised for the cytomorphological classification of acute leukaemia in adults based on May-Grünwald-Giemsa stained films of marrow and peripheral blood. Cytochemistry (Sudan Black and periodic acid Schiff) often confirmed the diagnosis but never necessitated a revision. The notation is flexible and allows for the recognition and easy retrieval of small subgroups not accounted for by conventional nomenclature. The main subtypes are given numbers (M0 to M6) and small subgroups are denoted by letters. Thus granulocytic leukaemia showing early but almost exclusively eosinophilic differentiation is designated M2E. —J.H.

Control of Heparin Anticoagulation. Stewart, C. A. (1975). *Clin. Med.* 82 (12), 23.

The plasma thrombin clotting time was found to be a more reliable and sensitive index of heparin anticoagulation than the kaolin-activated partial thromboplastin time. —J.H.

Trout Ova, an Alternative Source of Anti-B. Rees, P., Cotton, R., Holt, P. D. J. and Anstee, D. J. (1975). *Med Lab Sciences* 33 13

A specific Anti-B agglutinin is found in the eggs of the brown trout *Salmo Trutta*. This anti-B may be used in routine ABO grouping and a system has also been developed in which trout anti-B may be used in automated blood grouping machines. This anti-B may not be used with enzyme treated cells. —L.M.M.

Effects of Mixing Speed and Duration on Haemagglutination Titres. Sutton, W. W. and Whittington, R. O. (1975). *Med Lab. Sciences* 33, 83.

A technical note concerning a reversed passive haemagglutination system which was used to evaluate mixing speed and duration of mixing time. It was concluded from the information obtained that the mixing speed and duration affect microdilution titres. —L.M.M.

A New Zealand Family with i Members. Signal, T. and Booth, P. B. (1975). *Vox Sang* 30, 391.

A New Zealand (Caucasian) family with two i brothers was discovered by an anomalous routine blood grouping reaction. The i propositus and the individual whose anti-i caused the discovery were both voluntary blood donors of long standing. —Author's Summary

A New Genetic Determinant of γ G3⁺. Borel, H., Alper, C. and Schier, P. H. (1975) *Vox Sang* 30, 321.

A new antigenic determinant Ry confined to the γ G3 sub-group is described. Ry is present in almost all normal human sera of all GM phenotypes. —L.M.M.

Utilisation of Red-Cell Blood Groups in Gene Mapping: a Review. Marsh, W. L. (1975). *Med Lab. Sciences* 33, 125.

Blood group genes are clearly expressed and their products are readily detectable—this makes them excellent markers for use in gene mapping of the chromosomes. Studies of a patient whose red cells exhibit mosaicism for the Rh blood group, indicate that loss of the end of the short arm of the number 1 chromosome is associated with loss of an Rh gene complex. The Rh blood group gene can be assigned with some precision to this chromosomal location. —L.M.M.

Microbiology

Direct Immunofluorescent Test for the Detection of Gonorrhoea. Klanica, J. and Stejskalová, M. (1976). *Brit. J. ven. Dis.* 52, 33.

In examining 1100 women with chronic gynaecologic complaints the direct fluorescent antibody test proved to be more sensitive and less expensive than traditional cultural techniques. —J.H.

Chlamydial Infection of the Male Urethra. Oriel, J. D., Reeve, P., Wright, J. T. and Owen, J. (1976). *Brit. J. ven. Dis.* 52, 46.

Urethral specimens from 477 men were collected with endourethral swabs and examined for *Chlamydia trachomatis* by cell culture on McCoy cells pre-treated with idoxuridine. Of these men, 141 had gonococcal urethritis, 262 non-gonococcal urethritis (NGU), and 74 showed no evidence of urethritis. Of 118 men with heterosexually acquired gonococcal urethritis, 30 (25%), and of 23 men with homosexually acquired gonococcal urethritis, 5 (22%) yielded *C. trachomatis* from the urethra. *C. trachomatis* was recovered from the urethra in 3 (5%) of 60 heterosexual men without urethritis, and none of 14 homosexual men without urethritis yielded *C. trachomatis*. Improved methods of specimen collection and cell culture indicate that *C. trachomatis* is associated with approximately one-half of NGU infections.

In the authors' Department, this organism is now the commonest pathogen recovered from the male urethra. —J.H.

Control of Cost and Medical Relevance in Clinical Microbiology. Bartlett, R. C. (1975). *Amer. J. clin. Pathol.* 64, 518.

An increasing workload and decreasing funds available to carry out bacteriological work led Dr Bartlett to critically examine the work of his Microbiology Department by assessing the quality of incoming specimens and rejecting those which were of poor quality and limiting the work on those which were of dubious quality. Dr Bartlett claims to have reduced the bacteriology laboratory costs by 12% while handling an increased workload. This paper is

of interest to anyone concerned with Quality Control and the cost of microbiology laboratories.

—D.G.B.

Post Pneumonectomy Empyema due to *Neisseria mucosa*. Thorsteinsson, S. B., Minuth, J. M. and Musher, D. M. (1975). *Amer. J. clin. Pathol* **64**, 334.

A rare case of undoubted clinical infection due to this normal inhabitant of the upper respiratory tract is described. Full bacteriological details are given.

—D.G.B.

Survey of Clinical Microbiological Techniques used in the United Kingdom. Hamilton, W. J. and Martin, R. J. (1975), *Med. Lab. Technol.* **32**, 307.

The results of a survey of microbiological techniques used in the United Kingdom are described. The paper is well worth reading by those who are interested in microbiological techniques and in Quality Control. It is interesting to note that there is a very wide variation in the techniques used for the isolation of faecal pathogens and a very limited use of the newer types of medium. It would be interesting to see a similar enquiry in New Zealand.

—D.G.B.

Transportation Delay and the Microbiological Quality of Clinical Specimens. Jefferson, H., Dalton, H. P., Escobar, M. R., and Alison, M. J. (1975), *Amer. J. clin. Pathol.* **64**, 689.

This paper examines and compares the differences in the number of microbiological species isolated from replicate cultures inoculated directly on to culture media in the ward immediately after collection and of specimens which were inoculated on to culture media after transportation to the laboratory. Both unpreserved specimens and those held in various transport media were included in the transported specimens. Not surprisingly those specimens which were inoculated in the ward showed a considerably higher rate of pathogens, particularly of the more fastidious type. Some quite surprising differences in the efficiency of certain proprietary transportation media were revealed by this study. The authors make a rather surprising suggestion that all specimens should be inoculated in the ward. This would present problems which seem to this reviewer insuperable.

—D.G.B.

Methods for the Isolation of Anaerobes in the Clinical Laboratory. Dowell, V. R. (1975), *Amer. J. med. Technol.* **41**, 401.

A useful review of methods of isolation complete with details of culture media is given.

—D.G.B.

Identification of Anaerobes in the Clinical Laboratory. Holdeman, L. V. and Moore, W. E. C. (1975). *Amer. J. med Technol.* **41**, 411.

This brief review by Drs Holdeman and Moore attempts to compress the more comprehensive exposition of their methods for the identification of anaerobic species given the VPI anaerobe manual. The paper is however useful for those who do not have access to the manual.

—D.G.B.

Survival of Anaerobic Bacteria during Transportation. Justsen, T. and Nielson, M. L. (1976), *Acta Pathol. and Microbiol. Scandinavia.* **84B**, 51.

The authors aim to investigate transport of clinical material for anaerobic culture. Using known anaerobes an attempt is made to show variable recovery rates after flushing sample tubes with CO₂ and N₂. They conclude that a transport time of less than one hour gives a good recovery, pointing out that before flushing be recommended its benefits be investigated further.

—N.L.

Urine Culture: A new approach. Freidel, D., Amortegui, A. J. and Feinberg, S. (1975). *Amer. J. Med. Tech.* **41**, 454.

Pour plating is compared with a method involving the streaking of a standard inoculum with a nichrome wire loop. Although one is shown to be as good as the other the latter is considered more convenient.

—N.L.

A comparison of three readily available types of Anaerobic Blood Culture Media. Szawatkowski, M. V. (1976), *Med Lab. Sciences.* **33**, 5.

Three groups of media are investigated: Thiol broth, Thioglycollate, cooked meat and glucose broth. Results indicate that Thiol broth was the best media for the cultivation of anaerobes. A claim that Thiol broth was able to inactivate antibiotics is also investigated, and this was found to be the case for the antibiotics against which the broth was tested.

—N.L.

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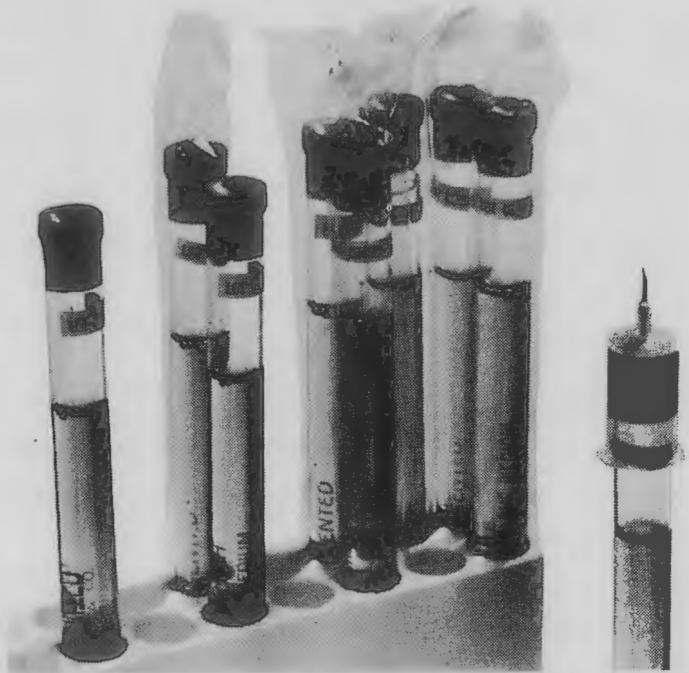
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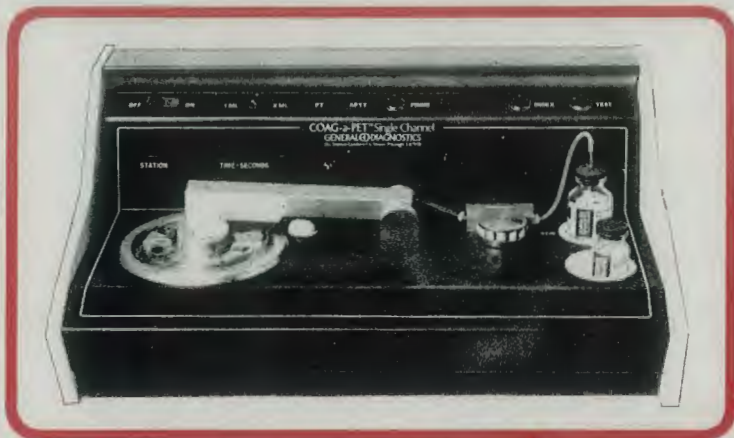
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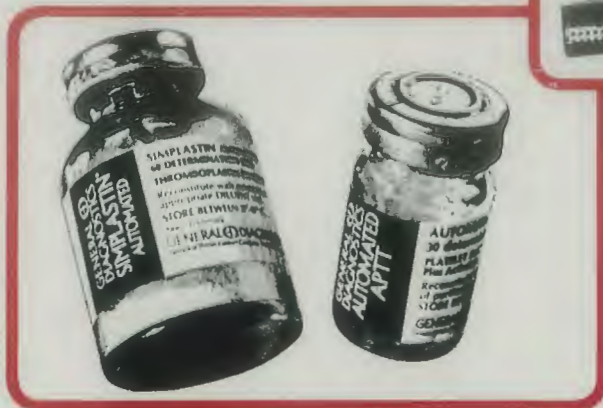
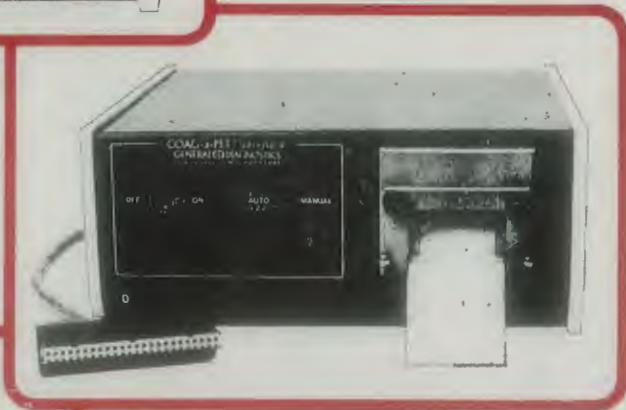


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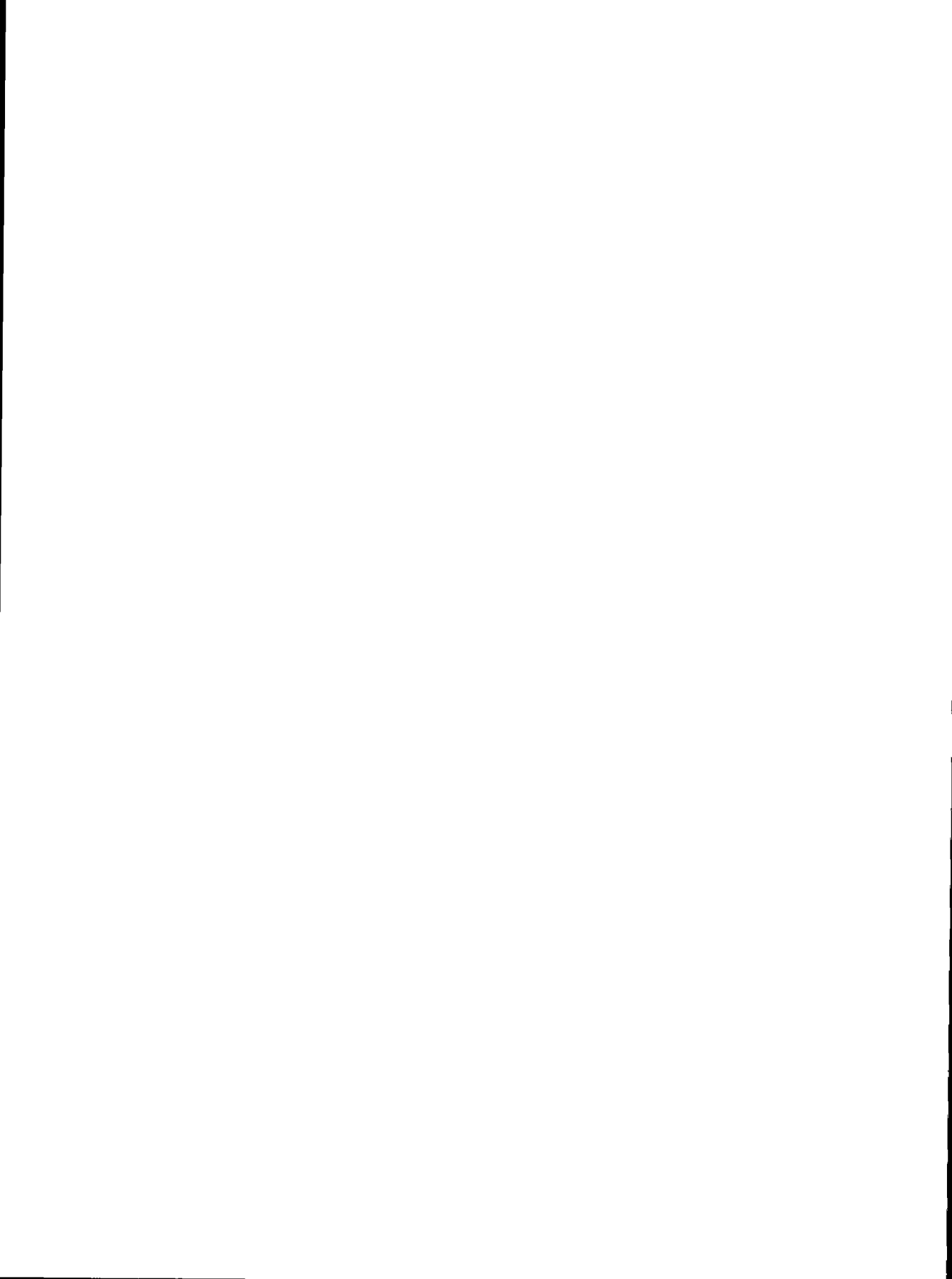
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