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JULY, 1974

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Reflections on Registration

The Medical Technologists' Regulations came into force on the first day of April 1973 and since then there has been time to reflect on their implications.

Registration is essentially a safeguard for the public, indicating that persons undertaking work involving the welfare of the community are competent to do so. The Statutory Board lays down the requirements for training and qualification and maintains discipline. In our case the essential qualification is the certificate of proficiency in hospital laboratory practice. All staff in medical laboratories do not possess this qualification or aspire to it. Laboratory assistants act in a supportive rôle and have their own qualifications. Their duties are generally well defined. Science graduates of varying description are also employed in the service and their position can appear to be anomalous. Some have expressed themselves rather heatedly, imputing disregard or neglect on the part of those responsible for compiling the regulations. Others however have taken the view that registration would result in loss of status as a scientist. To place the matter in perspective it is as well to consider the background to registration. The initial suggestion came from the Health Department. The relatively large number of medical laboratory technologists and the part they played in the hospital service merited this course.

Another pragmatic reason advanced was that it would facilitate administration of the examination system and related matters, as the governing body would have official standing. This course was first mooted in 1968 and met with a mixed reception but a referendum showed that most members were in favour of the idea. However five years of negotiating in the face of some opposition was required before registration became a fact and we were left in a position to manage our own affairs.

This was the culmination of 50 years' work and effort, usually uphill. In 1945 when the precursor of our professional body was set up there were only 24 financial members and the fact that there are now some 1,300 indicates the enormous increase in medical laboratory work since that time. We have again largely by our own efforts initiated educational and training regimes to keep up with the changing demands of medical laboratory technology. We might claim to have worked our passage.

The Medical Technologists' Regulations correctly recognise the predominating rôle of the medical technologist in the hospital laboratory service.

Provision is made for the trainee technologist who enters the service with a science degree. This is not the usual mode of entrance and is usually a matter of chance. However, people who enter in this way are invariably intelligent and excellent material for training. Vocational training is prescribed and modifications to this, particularly in regard to specialisation, are currently being discussed. On the other hand, the orthodox training is designed for the job. It is soundly based on science subjects leading to laboratory practice in the main disciplines. The theoretical work is carried out in parallel with practical instruction.

Entry to medical laboratory work is competitive and the entry standard is high. This all-round training confers many benefits; not only the ability to do several jobs in a small laboratory or the knack of consigning ambiguous requests to the correct department but in applying the techniques of associated disciplines to immediate problems. The type of person designated a senior hospital scientific officer normally requires a higher qualification in science and is said to be performing a task for which this qualification is required (or something to this effect): a rather quaint circular definition.

Curiously enough, there is no longer a junior version. This small but heterogeneous group performs a variety of tasks varying from essentially service laboratory work to that of a predominantly research nature. The Medical Technologists' Regulations do not apply to them except in the negative sense implied by regulation 12 subsection (1) (a). The sense of this is that a person is not prohibited from working in a medical laboratory as long as he does not hold himself out to be a registered medical technologist.

We live at some remove both spatially and temporally from the large industrial nations and one advantage is that the ripples of change and development take a little time to impinge upon the shores of our ministate. We

can see what other people do under similar circumstances.

Clinical biochemists, physicists, and microbiologists have long been accepted as a separate group or groups of laboratory staff overseas. These designations seem to command sufficient respect to preclude the need for those possessing them to be registered as medical technologists.

Is this not a matter for constructive thought and action on the part of those concerned? Manipulation of the spade rather than other implements?

R.D.A.

Increased Accuracy and Precision in Screening for Urinary Mucopolysaccharides

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Based on a paper delivered to the NZACB Annual Conference, Auckland, August 1973; and received for publication, April 1974

Summary

Difficulties encountered in screening for urinary mucopolysaccharides by the quaternary ammonium turbidity test have been overcome by a careful control of the reaction conditions. Carrying out the reaction at pH 6.0 in a final electrolyte concentration of 0.25-0.35M gives an optimum absorbance of the quaternary ammonium-mucopolysaccharide complex when read at 560nm. The test has been put on a quantitative basis by means of a high (1.0 percent) final cetyltrimethylammonium bromide concentration to prevent interference by urinary glycoprotein. An internal chondroitin sulphate standard offsets the coprecipitation of other non-mucopolysaccharide material.

Introduction

Mucopolysaccharides (MPS) are important constituents of connective tissue. As part of their normal catabolism 2-15mg appear in the urine every 24 hours, the actual amount depending on age, size and sex (in decreasing order of influence). The five main MPS excreted are chondroitin-4-sulphate, chondroitin-6-sulphate, dermatan sulphate, heparin sulphate, and keratan sulphate. The chondroitin sulphates (CS) normally comprise 80-90 percent of the output.

Our main interest in urinary MPS lay in attempting to develop a test for breast carcinoma dependent on changes in the normal balance of excreted MPS. This investigation is based on our earlier findings (Adams and Meaney, 1961)[1] of high levels of sulphated MPS occurring in breast tumour tissue, with dermatan sulphate predominating. Due to the enthusiasm of New Zealand paediatricians and geneticists, most of our efforts in this field have been devoted to isolating and estimating urinary MPS from specimens forwarded for examination for gargoylism. There are six major sub-types of the genetic mucopolysaccharidoses clearly established[6]. They are all characterised by a deficiency of one or more MPS-degrading enzymes[12] resulting in a tissue accumulation of MPS leading to structural deformity with occasional clouding of the cornea and impaired intellect. The conditions are invariably accompanied by an increased excretion of MPS in the urine that is characteristically abnormal in pattern. Chemical identification of the excreted MPS often enables final diagnosis of the sub-type.

As the MPS are highly charged polyanions, extensive use has been made of quaternary ammonium (QN^+) compounds to precipitate them quantitatively. However, the time taken to isolate, partly purify, and analyse a 24 hour yield is one week. It is not surprising that various attempts have been made to develop rapid screening tests.

A paper spot test employing the metachromatic reaction between sulphated MPS and toluidine blue[2] is prone to false positive results and, at best, is only semi-quantitative. McKusick (1960)[7] adapted the reaction between MPS

and acidified albumin[4] for detecting urinary MPS, but it suffered a lack of sensitivity and poor reproducibility. Denny and Dutton (1962)[3] further improved the method, but it has been reported to give false negative results[9] with a Hurler's syndrome specimen.

For rapid testing, greatest use has been made of the turbidity formed by MPS when cetylpyridinium chloride (CPC) or cetyltrimethylammonium bromide (CTAB) are added to urine[8, 10, 11]. Such tests using CPC or CTAB also have considerable limitations, being prone to false positive and, less commonly, false negative results. These faults are due, respectively, to interfering QN^+-precipitable urinary material, and failure to allow for variations in urine pH and electrolyte levels.

Our investigations have shown that there are six main factors which affect the absorbance of the subsequent MPS-QN^+ complex when carrying out screening tests on urine with QN^+ compounds. These are:

1. The wavelength for reading the absorbance of the complex.
2. The electrolyte level of the reaction.
3. The pH of the reaction.
4. The time of reading the absorbance.
5. The presence of both protein and other interfering materials.
6. The completeness of a 24-hour collection.

Pennock (1969)[10] recognised the influence of ionic strength and pH on the turbidity test and sought to stabilise them by carrying out the reaction at pH 4.8 in 0.05M citrate buffer. These conditions do not exclude the effect of non-MPS interfering material and gave low results with dilute urine samples. It has also been found in our laboratories that neither citrate nor acetate are electrolytes of choice for carrying out QN^+ precipitation reactions.

None of the earlier screening tests were capable of producing a quantitative result for MPS excretion. It is proposed to show that, by careful control of the above factors, a precise and reasonably accurate estimate of urinary MPS excretion may be obtained.

Materials

1. 2.0N HCl
2. 2.0N NaOH
3. 1.0M NaCl
4. Stock standard (1.0mg/ml) CS. (Sigma Grade II mixed chondroitin sulphate isomers.) Stored frozen.
5. Working standard (100μg/ml) CS; a 1 in 10 dilution of the stock standard. Also stored frozen.
6. 5.0 percent CTAB (BDH laboratory reagent). Stored at 37°C. May require filtering before use (this is important).
7. Urine sample.

The volume is noted, and the pH adjusted to pH 6.00 using the 2.0N HCl or NaOH. A small sample is removed for analysis of creatinine, sodium, and potassium. The remainder is stored frozen overnight, if possible thawing and re-freezing several times. Prior to assay, the specimen is thawed and an aliquot clarified by centrifuging at 3000r.p.m. for 15 minutes.

Method

1. To each of three tubes labelled Blank, Test, and Internal Standard, 0.3ml of 1.0M NaCl is added.*
2. To the Internal Standard tube, 0.2ml (i.e., 20μg) of working standard CS is added.
3. 1.0ml of the pH-adjusted, clarified urine is added to each tube.
4. Water is added at the rate of 1.2, 0.7, and 0.5ml to the Blank, Test, and Internal Standard tubes respectively.*
5. 0.5ml of 5.0 percent CTAB is added to the Test and Internal Standard tubes only.
6. The contents of each tube are thoroughly mixed and allowed to stand for exactly 15 minutes at room temperature.
7. The absorbancies of each tube are read at 560nm against water.
8. The MPS levels are calculated as follows:

$$\frac{T\text{-}B}{IS\text{-}T} \times 20 = \mu g \text{ MPS/ml urine}$$

Where B, T, and IS are the absorbancies of the Blank, Test, and Internal Standard respectively.

9. From the result of the urinary creatinine analysis, MPS excretion is converted to μg/mg creatinine as follows:

$$\frac{T\text{-}B}{IS\text{-}T} \times 20 \times \frac{100}{C} = \text{MPS/mg creatinine}$$

Where C = mg creatinine/100ml urine.

* *Footnote: Both added NaCl and water volumes are varied if the combined sodium and potassium concentrations of the pH-adjusted urine lie outside of the range 0.15-0.30M. For a combined sodium and potassium level of less*

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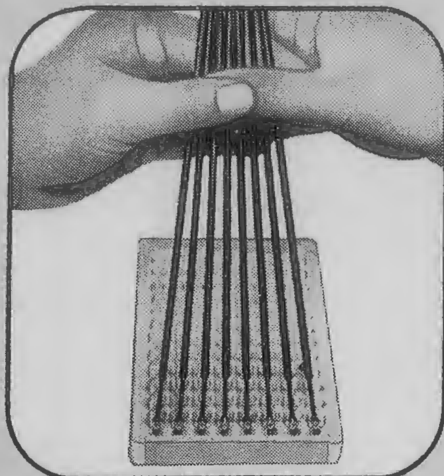

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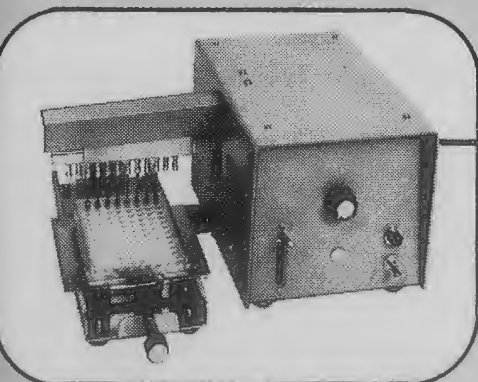

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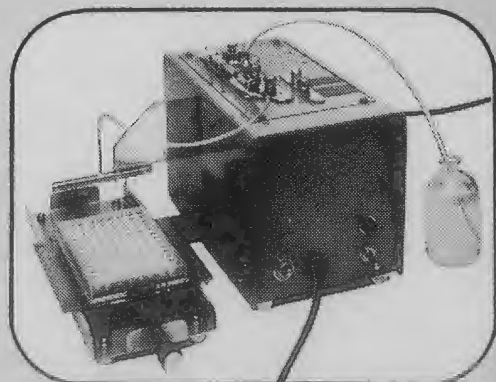

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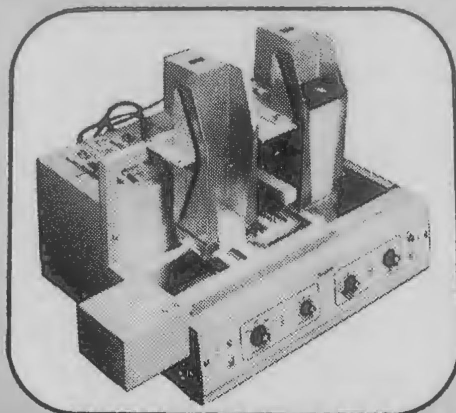

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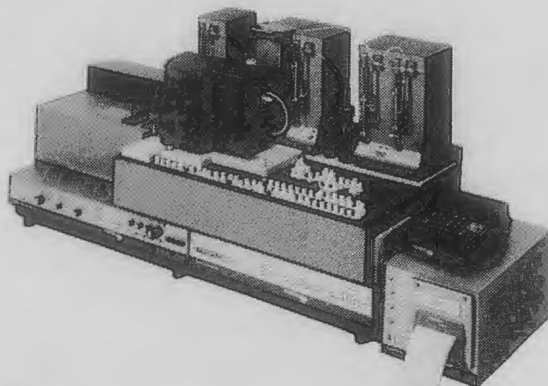

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Results

1. Choice of wavelength—Reading the turbidities of the MPS-QN^+ complex at 560nm was found to give a maximum absorbance for the complex, consonant with minimum interference from urinary pigments.

2. The effect of electrolyte—Figure 1 shows the effect of increasing concentrations of added NaCl on the absorbance of the CS-CTAB suspension.

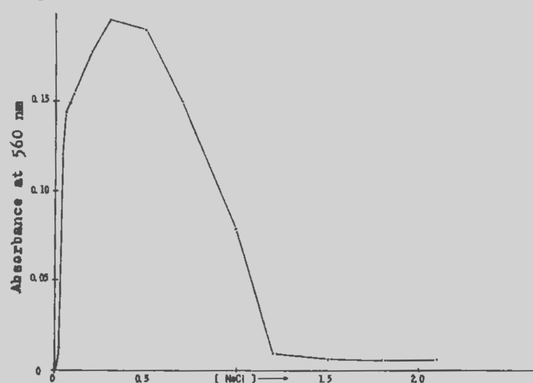

Figure 1: *The effect of increasing concentrations of NaCl on the absorbance of the complex formed between chondroitin sulphate (10μg/ml) and 0.1 percent cetyltrimethlyammonium bromide.*

3. The effect of pH—As the pH of the precipitation reaction was increased above the pK of the acidic groups on the MPS, so the absorbance of the QN^+-complex increased. Similarly, as the pH rose above the pI of urinary proteins, these became increasingly anionic and began to precipitate heavily. pH 6.00 was found to be optimal for obtaining the maximum MPS-QN^+ absorbance consistent with the least interference from other reacting materials.

4. Incubation time—Table I shows that the maximum absorbance of a complex formed by CTAB and an external CS standard was achieved in over two hours. However, when the same CS was added to urine, as an internal standard, the absorbance rapidly reached a peak and declined after 15 minutes. This was due to the flocculation of interfering material in the urine.

than 0.15M, 0.4ml of 1.0M NaCl is added, with 0.1ml less of water. For combined molarities exceeding 0.30M, 0.2ml of the NaCl is used, together with an additional 0.1 ml of water.

Table I: The effect of time of incubation on the formation of mucopolysaccharide-cetyltrimethylammonium bromide complexes.

Time (min.)	*Absorbance 560nm of CS standard* Internal	External	*Urinary MPS* (mg/g creatinine)
0	0.097	0.028	32.5
3	0.177	0.076	37.0
6	0.204	0.080	38.1
9	0.214	0.092	39.0
15	0.238	0.096	36.1
30	0.226	0.108	39.8
60	0.221	0.126	43.6
120	0.205	0.141	47.6
420	0.138	0.124	58.4

From parallel blank and test readings taken at the same times, the concentration of urinary MPS was calculated. As seen from Table I the results obtained were fairly constant (38.0 ± 1.5mg MPS/g creatinine) over the time range 3-30 minutes.

The same table illustrates the extent of non-polysaccharide interference. Both the external and internal standard employed added CS to a final level of 8.0μg/ml. The absorbance for the latter was considerably higher due to the co-precipitation of interfering material. Analysis of non-MPS material isolated from urine showed that up to 120mg of CTAB-precipitable protein was present in 24 hour urine collections from children. A scaled-down Biuret method was used; this protein generally escaped detection by the heating and automated sulphosalicylic acid techniques.

5. The effect of QN^+ concentration—(1) *On authentic CS*—Two levels of CS standard solution (10μg/ml and 60μg/ml) equivalent to moderate and high normal levels of urinary MPS, respectively, were precipitated in the presence of 0.2M NaCl with increasing concentrations of CTAB over the range 0-2.0 percent (a practical upper limit imposed by CTAB solubility). With 10μg/ml CS, a linear 33 percent increase in the absorbance of the CS-CTAB complex occurred over the range 0.05-2.0 percent CTAB. A linear 28 percent increase in turbidity was found for the 60μg/ml CS, over the same range of CTAB concentrations.

(2) *On urine*—The urine of a normal male 5-year-old child was used in the experiment to exploit the high urinary glycoprotein content

and MPS level (49μg/ml in this case) commonly found in this age group. Reactive urine components were precipitated, in the presence of approximately 0.3M total electrolyte, with increasing concentration of CTAB over the range 0-2.0 percent. The absorbancies of the urine-CTAB complex declined markedly (by more than 50 percent) over the range 0.05-2.0 percent CTAB. The decrease was not linear, most occurring as the CTAB concentration rose from 0.05-0.20 percent. The absorbance of the urine-CTAB complex levelled off at approximately 1.0 percent CTAB concentration.

When exogenous CS was added as an internal standard, the absorbance due to the added CS alone was found to increase by about 20 percent over the same range of CTAB concentrations.

Figure 3, later, illustrates the consistently lower values for urine MPS excretion over a 24-hour period obtained by the screening test using a final CTAB concentration of 1.0 percent. as compared with the results employing 0.1 percent CTAB.

6. The effect of cold-storage of urine—The MPS screening test gave a higher reading for fresh urine than on the same sample which had been stored cold or frozen overnight. Figure 2 illustrates the effect of time of cold storage on the calculated urinary MPS levels of three different urines.

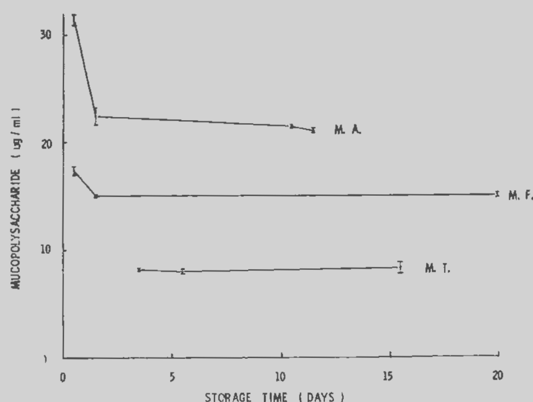

Figure 2: *The effect of cold storage of urine on their apparent mucopolysaccharide levels, calculated from the cetyltrimethylammonium bromide screening test.*

7. Accuracy of the turbidity test—Table II compares the values for urinary MPS calculated by the screening test with the values obtained by completely isolating and analysing the MPS from the same series of child urines.

Table II: Comparison of urinary mucopolysaccharide excretion in children by means of the turbidity test and chemical analysis.

mg MPS/g creatinine/24 hours

Subject	*Sex*	*Age*	*Condition*	*Turbidity test*	*By analysis*
M.A.	M	5	Normal	28.3	23.0
I.M.	M	9	Normal	22.8	20.9
G.S.	M	11	Query	20.3±3.5(3)	18.2±2.4(3)
A.R.	M	4	Hurler	314	282
S.T.	F	6	Morquio	38.9	42.9*

* This value includes 21.9mg of keratan sulphate.

Table III compares the screening test results for a number of adult urines with the analytical results from the isolated MPS.

Table III: Comparison of urinary mucopolysaccharide excretion in adults by means of the turbidity test and chemical analysis.

mg MPS/g creatinine/24 hours

Subject	*Age*	*Condition*	*Turbidity test*	*By analysis*
Male				
M.M.	43	Normal	6.1	6.2
L.W.	61	Polychondritis	7.6	6.7
Female				
R.M.	31	Normal	14.4	16.4
Various	—	Ca. of breast	17.0±13.8(5)	16.5±12.2(5)

8. Diurnal variation in MPS excretion—Individual voluntary urine collections were taken over 24 hours from four normal male children, ages 5 to 12 years. MPS excretion was examined by the screening test and calculated as mg/hour. All subjects showed a common decline in MPS excretion in the middle of the day followed by a steady rise to a late afternoon or early evening peak.

Creatinine excretion, also calculated as mg/hour, followed a similar but less marked pattern. The number of separate collections over 24 hours ranged from 3-7.

The study was expanded with a further four subjects, this time eschewing voluntary collection and increasing the frequency of sampling to approximately hourly intervals during wakefulness, as well as obtaining two additional samples during their normal hours of rest. A better resolution of diurnal variation was achieved, showing a common nadir in MPS excretion around 1.00 p.m. In addition, a similar 1.00 a.m. decrease was uncovered when sufficient samples were taken.

Creatinine excretion again showed a parallel pattern of diurnal variation.

Figure 3 illustrates a typical pattern of excretion of MPS and creatinine exhibited by a normal 9-year-old child.

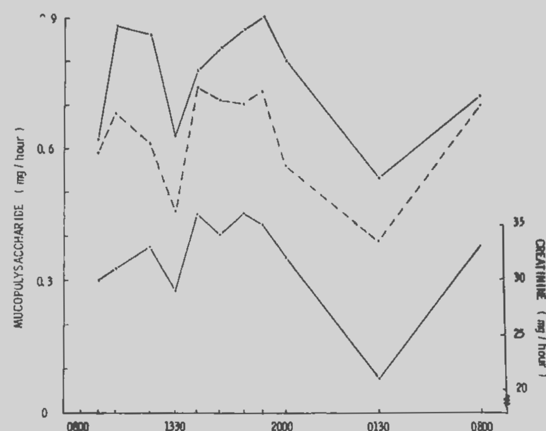

Figure 3: *Diurnal variation in urinary mucopolysaccharide and creatinine excretion in a normal male child. The broken line denotes true MPS excretion. The upper unbroken line denotes values of MPS excretion obtained by carrying out the turbidity test with a low (0.1 percent) CTAB concentration. The lower unbroken line denotes creatinine excretion.*

Discussion

The results show the necessity for close control of the pH and electrolyte levels of the MPS-QN+ turbidity reaction, in order to ensure precision. At pH 6.0, the maximum absorbance of the complex was obtained, with the least interference from the precipitation of other material. Most urine samples were found to have a combined sodium and potassium concentration in the range 0.15-0.30M, after adjusting the pH. Addition of the recommended level of NaCl (0.3ml of a 1.0M solution) will bring the molarity of the turbidity reaction to between 0.25-0.35M, when the contribution of other urinary cations is included. Reference to figure 1 will show that this is the optimum range for visualising the CS-CTAB complex. Although both CTAB and CPC have similar optimum electrolyte ranges, and give identical absorbancies with the same level of MPS, the former is preferred as CPC "salts out" with high concentrations of NaCl.

One of the main factors causing errors in the turbidity test and preventing its development as a quantitative test has been the marked interference by co-precipitating material. The extent of this interference may be seen in Table I on comparing the absorbancies of reactions using the same amount of added CS as an internal and an external standard. This interference has been overcome in two ways. Firstly, the CTAB level of the reaction was raised to 1.0 percent. This gave the twofold advantage of increasing the absorbance of the MPS-CTAB complex, presumably by a mass action effect, and of reducing the level of precipitated glycoprotein.[5]. Figure 3 compares the MPS levels of a series of urine samples calculated from tests using both 0.1 percent and 1.0 percent final CTAB concentrations. Secondly, the added internal standard apparently attracts the same level of coprecipitating material as does the endogenous urinary MPS. Finally, although the time of reading the test appears to be fairly elastic, from 3-30 minutes, it is essential that all components of the one assay (Blank, Test, and Internal Standard) be read after standing exactly the same length of time. Fifteen minutes appears to be a suitable incubation period.

Tables II and III illustrate the results for a series of child and adult urine, calculated from the screening test and compared with the yields obtained by isolation and analysis of the urinary MPS. In a survey of 24-hour urine specimens ($n = 23$) the screening test gave results 6.9 ± 7.8 percent higher than obtained from the full recovery of the constituent MPS. Comparison by the one-tailed students t test showed that both results belong to the same population, thus establishing that the modified screening test assures a reasonably accurate representation of the true urinary MPS content.

Difficulties were encountered in obtaining full 24-hour collections due to the youthfulness or impaired intellect of some of the subjects. To explore the validity of spot tests, carried out on random urine samples, a study of diurnal variation in MPS excretion was made. A marked and fairly standard circadian rhythm was found. This was paralleled by a similar but somewhat less marked pattern in creatinine excretion, as illustrated in Figure 3. Relating the MPS to creatinine excretion, as mg MPS/g creatinine, gave a fairly constant measure of MPS output.

Urines for testing are best preserved by cold or frozen storage, with the advantage that some interfering material is precipitated (see Figure 2). The use of solvents, toluene or chloroform, should be avoided.

Acknowledgments

Messrs E. Johnson and T. Wilson of the Auckland Hospital Clinical Laboratory are

thanked for providing precise urinary creatinine and electrolyte analyses.

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Obituary

E. L. F. Buxton

With the death of Ernest Laurence Fletcher Buxton at the age of 74, following a road accident earlier this year, the Institute has lost one of its important foundation members.

It was, however, as Laurie Buxton that we all knew him. He carried his age so well that we tended to forget that he commenced work in 1917 in the Wallaceville Veterinary Laboratory and transferred for training as a medical bacteriologist to the Wellington Hospital Laboratory in 1920. He later moved to the Auckland Hospital Laboratory and in 1928 was appointed to the position of bacteriologist to the Wanganui Hospital. It was in this position that he exercised his particular laboratory talents so that by the time he retired from that position in 1955, he left a most substantial and modern laboratory.

From the point of view of the members of the Institute however, he should be remembered for his work in getting together the group of medical technologists who formed the precursor to the Institute. It was his tact and vast experience of committee work which welded together the disparate views of the technologists from all parts of New Zealand and which led to the formation of the New Zealand Association of Bacteriologists with himself as the first president. This was a hard working, completely united body which with only 75 members was able to make such forceful representations to the Government that by the second conference in 1946 it was recognised by the pathologists, the Hospital Boards' Association and by the Department of Health as speaking for the medical technologists of New Zealand.

This was no chance matter and owed much to the patently honest character of Laurie Buxton. This was, of course, entirely in character as he was a sincere and practising Christian, who as a Methodist held almost every office open to him as a layman in that Church. Furthermore he was President of the Wanganui Crippled Children Society for many years.

As a man he was a good friend and one of whose honesty there was never any doubt. He fought doggedly and with great courage for those principles which he held. The Institute is greatly in his debt for creating the image of the technologist as a sensible, logical, and reasonable being but one who could not lightly be put upon.

In Laurie Buxton we recognise a hard working life, well spent. To his widow and his family we offer our sympathy and our belief that he and his works will be long remembered.

—D. W.

Normal Haematological Values in the Waikato, New Zealand

E. Oudyn, ANZIMLT

Haematology Department, Hamilton Medical Laboratory

Received for publication, March 1974

Summary

The haematological parameters of approximately 12,500 apparently healthy people, approximately 100 from each sex and age group from one to seventy, were measured to establish a normal range. The parameters included WBC, RBC, Hb, HCT, MCV, MCH, and MCHC. The data for this paper were collected over a two-year period.

Introduction

With the advent of automation in haematology, namely the Coulter Model S, many results can be obtained to establish the normal ranges of the various haematological parameters. The computer makes the processing of this information a relatively simple matter. Up to the present time the normal values used in this laboratory were those from Practical Haematology by J. V. Dacie and S. M. Lewis, the fourth edition. Some of the values obtained in this paper differed from these as did the methods used for determining the various parameters. The selection of individuals used in this survey was on the basis of the blood film appearance and clinical particulars where supplied.

Materials and Methods

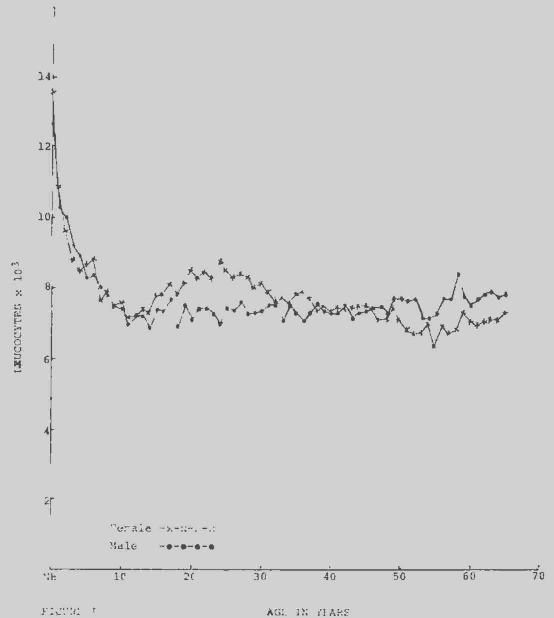
The majority of the blood samples were collected by vein puncture and delivered into EDTA. Heelpricks were done on a number of the infants and children. All parameters were measured on the Coulter Model S. The results were put into age groups and punched on punch cards and then processed on an IBM 1130 computer.

Results

Because of the large numbers involved it was decided to summarise results by plotting the mean values of each age group and sex on a graph. See Figures 1 to 7. Normal ranges were arranged into five-year groups the results of which may be seen in Tables I to VII. Results of individual ages are available on request.

Leucocyte Count

Leucocyte values may be a little low due to the fact that some of the EDTA specimens were 24 hours old before being processed, this being due to the delay involved in posting specimens from outlying areas. Mean values in childhood went from 14,000/cmm for the newborn to 7,000/cmm at puberty. No appreciable differences were found between males and females. Females in the 15 to 35 year age group showed a rise during childbearing years and for some unexplained reason male values were higher than females in later years.



AGE IN YEARS

TABLE 1

LEUCOCYTE COUNT

Age Group	MALES			FEMALES		
	No in Group	Mean ± 2 S.D.	Mean	No in Group	Mean ± 2 S.D.	Mean
Cord Blood	105	6.6 - 19.6	12.8	96	7.0 - 20.4	13.7
1 - 5	463	3.7 - 14.7	9.2	385	3.9 - 14.3	9.1
6 - 10	522	2.9 - 12.7	7.8	470	3.1 - 12.6	7.9
11 - 15	488	2.9 - 11.4	7.1	484	3.3 - 11.4	7.4
16 - 20	455	3.3 - 11.3	7.3	496	3.7 - 12.5	8.1
21 - 25	499	3.3 - 11.3	7.3	561	4.1 - 12.9	8.5
26 - 30	441	3.0 - 11.8	7.4	498	3.9 - 12.5	8.2
31 - 35	450	3.2 - 11.5	7.4	528	3.4 - 12.1	7.8
36 - 40	431	3.1 - 11.6	7.3	476	3.5 - 11.6	7.6
41 - 45	444	3.3 - 11.4	7.3	488	3.7 - 11.2	7.5
46 - 50	470	3.6 - 11.4	7.5	462	3.4 - 11.0	7.2
51 - 55	343	3.7 - 11.1	7.4	473	3.2 - 10.3	6.7
56 - 60	402	3.7 - 11.8	7.7	398	3.5 - 10.5	7.0
61 - 65	329	3.8 - 11.8	7.8	368	3.4 - 10.8	7.1
66 - 70	240	3.8 - 11.5	7.7	318	3.6 - 10.6	7.1

Red Cell Count

Before the advent of electronic cell counters the red cell count was not considered an accurate parameter. Now however, with an accuracy of ± 2 percent it is an extremely useful index especially when taken together with the other indices. Acute blood loss, megaloblastic anaemias, thalassaemia, polycythaemia, and even iron deficiency anaemia can be readily indicated in this manner and can then be confirmed by blood film examination and other tests.

Dacie's range of 4.5 to 6.5 million per cmm for males and 3.9 to 5.6 million per cmm for females was found to be a little wide. Our findings showed male values of 4.4 to 5.8 million per cmm and 3.6 to 5.2 per cmm for females in the childbearing period and 3.9 to 5.4 post-menopausal.

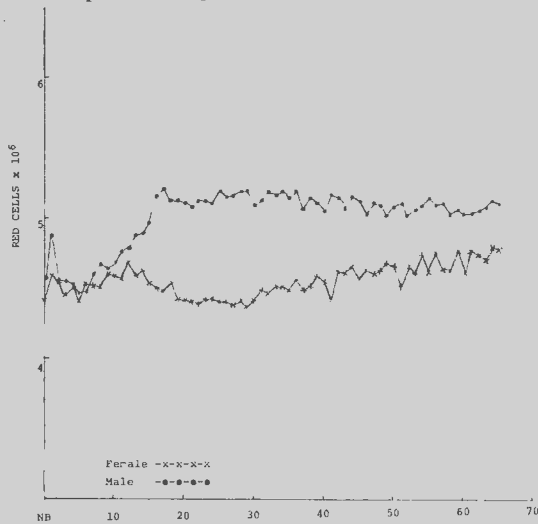


FIGURE 2 AGE IN YEARS

TABLE 11

RED CELL COUNT /cm

Age Group	MALES			FEMALES		
	No in Group	Mean ± 2 S.D.	Mean	No in Group	Mean ± 2 S.D.	Mean
Cord Blood	105	3.7 - 5.5	4.6	99	3.5 - 5.4	4.4
1 - 5	463	3.7 - 5.3	4.5	385	3.8 - 5.2	4.5
6 - 10	522	3.9 - 5.[illegible]	4.6	470	3.9 - 5.2	4.6
11 - 15	488	4.1 - 5.5	4.8	484	3.9 - 5.3	4.6
16 - 20	455	4.5 - 5.8	5.1	496	3.7 - 5.3	4.5
21 - 25	499	4.4 - 5.8	5.1	561	3.6 - 5.2	4.4
26 - 30	441	4.4 - 5.8	5.1	498	3.6 - 5.2	4.4
31 - 35	450	4.4 - 5.8	5.1	528	3.7 - 5.3	4.5
36 - 40	431	4.4 - 5.8	5.1	476	3.8 - 5.3	4.5
41 - 45	444	4.4 - 5.8	5.1	482	3.7 - 5.4	4.6
46 - 50	470	4.3 - 5.8	5.0	462	3.9 - 5.4	4.6
51 - 55	343	4.3 - 5.8	5.0	473	3.9 - 5.4	4.6
56 - 60	402	4.3 - 5.8	5.0	398	3.9 - 5.4	4.7
61 - 65	319	4.2 - 5.8	5.0	368	4.0 - 5.4	4.7
66 70	240	4.2 - 5.8	5.0	318	3.9 - 5.4	4.7

Haemoglobin

Here again we see female values drop during childbearing years with a rise post-menopausally, however not quite reaching male values in later years. The range of male values varied from 10.0g to 14.0g in children to about 13.0g to 17.0g in adults. Adult female values ranged between 10.9g to 15.8g.

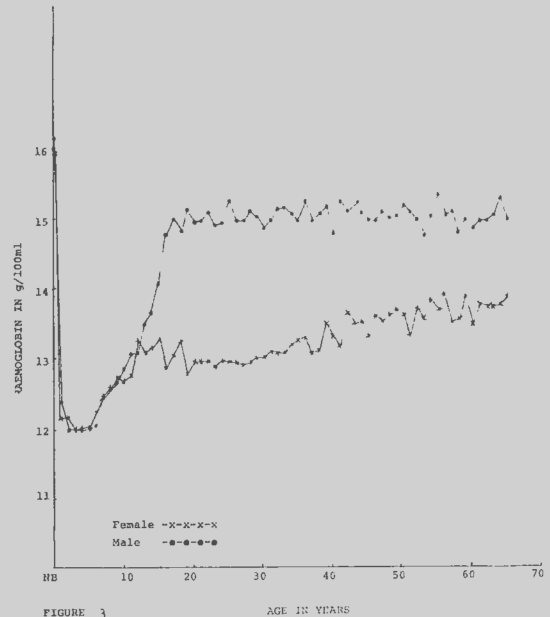


FIGURE 3 AGE IN YEARS

TABLE 111

HAEMOGLOBIN g/100ml

Age Group	MALES			FEMALES		
	No in Group	Mean ± 2 S.D.	Mean	No in Group	Mean ± 2 S.D.	Mean
Cord Blood	105	13.3 - 18.9	16.1	99	12.6 - 19.1	15.8
1 - 5	463	10.0 - 14.0	12.0	385	10.1 - 13.9	12.0
6 - 10	522	10.8 - 14.1	12.5	470	10.9 - 14.1	12.5
11 - 15	488	11.5 - 15.3	13.4	484	11.3 - 14.8	13.0
16 - 20	455	13.1 - 16.8	14.9	496	10.9 - 15.0	12.9
21 - 25	499	13.2 - 16.8	15.0	561	10.8 - 15.5	12.9
26 - 30	441	13.1 - 16.9	15.0	498	10.8 - 15.1	12.9
31 - 35	450	13.2 - 16.8	15.0	528	11.0 - 15.2	13.1
36 - 40	431	13.1 - 16.9	15.0	476	11.0 - 15.5	13.2
41 - 45	444	13.0 - 17.1	15.0	488	11.5 - 15.4	13.4
46 - 50	470	13.0 - 17.1	15.0	462	11.5 - 15.7	13.6
51 - 55	343	12.9 - 17.1	15.0	473	11.7 - 15.5	13.6
56 - 60	402	12.8 - 17.1	14.9	398	11.6 - 15.7	13.7
61 - 65	319	12.9 - 17.1	15.0	368	12.0 - 15.5	13.8
66 - 70	240	12.8 - 17.0	14.9	318	11.5 - 15.8	13.7

Haematocrit

Values for the haematocrit are calculated from the red cell count and the mean cell volume and as the haematocrit is not directly measured this could explain the slight difference in the normal values obtained from those previously used.

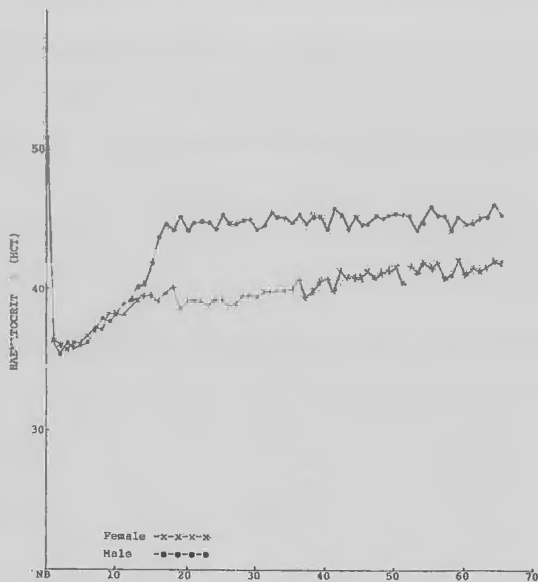


FIGURE 4 AGE IN YEARS

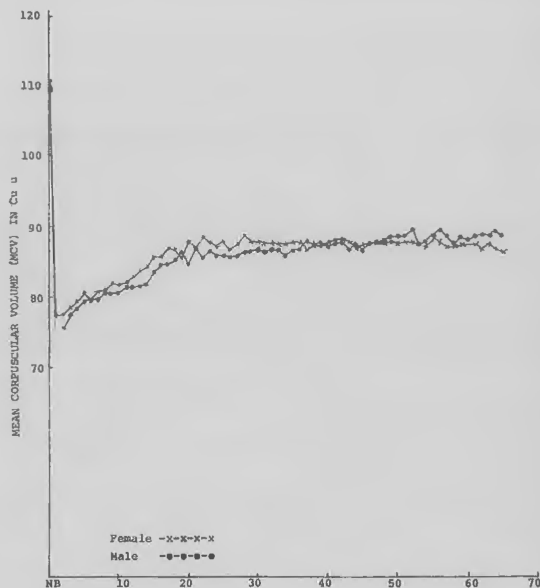


FIGURE 5 AGE IN YEARS

TABLE IV

HAEMATOCRIT

Age Group	MALES			FEMALES		
	No in Group	Mean ± 2 S.D.	Mean	No in Group	Mean ± 2 S.D.	Mean
Cord Blood	105	41.1 - 60.0	50.8	96	40.0 - 60.2	50.1
1 - 5	463	30.0 - 41.7	35.9	385	30.8 - 41.3	36.0
6 - 10	522	32.5 - 42.3	37.4	470	32.4 - 42.5	37.5
11 - 15	488	34.3 - 45.6	40.0	481	33.8 - 44.5	39.2
16 - 20	455	38.6 - 50.2	44.4	496	33.1 - 45.6	39.3
21 - 25	499	39.1 - 50.5	44.8	561	32.9 - 45.3	39.1
26 - 30	441	39.0 - 50.4	44.7	498	32.6 - 45.7	39.2
31 - 35	450	39.3 - 50.7	45.0	528	33.6 - 46.2	39.9
36 - 40	431	39.1 - 50.7	44.9	476	33.7 - 46.9	40.3
41 - 45	444	39.1 - 51.1	45.1	488	34.8 - 46.8	40.8
46 - 50	470	39.0 - 51.3	45.1	462	34.8 - 47.7	41.3
51 - 55	343	38.7 - 51.6	45.1	473	35.3 - 47.3	41.3
56 - 60	402	38.5 - 51.5	45.0	398	35.1 - 47.9	41.5
61 - 65	319	38.9 - 51.6	45.3	368	36.5 - 46.9	41.7
66 - 70	240	38.5 - 51.6	45.0	318	35.0 - 48.3	41.7

Mean Corpuscular Volume

Mean cell volume on the Coulter S is measured by volume displacement and is considered an accurate index. The range of 76 to 96cμ was found to be rather inadequate especially for the mean values of children. For example, our normal range was 69 to 87cμ for children in the 1 to 5 year age group. An MCV of 94cμ in this age group is automatically suspect and usually has some underlying cause. Up to the age of 40 female values are slightly in excess of male values for the same group after which male values show a slight increase over female values.

The results are summarised in Table V.

TABLE V

MEAN CELL VOLUME

Age Group	MALES			FEMALES		
	No in Group	Mean ± 2 S.D.	Mean	No in Group	Mean ± 2 S.D.	Mean
Cord Blood	105	95.5 - 122.8	109.1	99	98.5 - 123.2	110.8
1 - 5	463	68.9 - 86.3	77.6	385	69.8 - 87.9	78.8
6 - 10	522	72.6 - 87.7	80.1	470	72.8 - 88.8	80.8
11 - 15	488	74.1 - 89.3	81.7	481	75.5 - 91.5	83.5
16 - 20	455	77.9 - 92.6	85.22	496	78.6 - 94.2	86.4
21 - 25	499	78.5 - 93.6	86.1	561	79.2 - 75.6	87.4
26 - 30	441	77.9 - 94.1	86.0	498	79.2 - 76.0	87.6
31 - 35	450	78.5 - 94.4	86.4	528	78.2 - 96.6	87.4
36 - 40	431	79.3 - 95.2	87.3	476	78.5 - 96.2	87.3
41 - 45	444	79.4 - 95.1	87.3	488	78.8 - 96.3	87.5
46 - 50	470	80.0 - 96.2	88.1	462	78.8 - 96.0	87.4
51 - 55	343	79.5 - 97.4	88.5	473	79.1 - 96.0	87.9
56 - 60	402	80.3 - 96.5	88.4	398	79.0 - 95.9	87.4
61 - 65	319	81.0 - 96.8	88.9	368	79.1 - 95.1	87.2
66 - 70	240	80.0 - 96.7	88.4	316	79.1 - 96.0	87.6

Mean Corpuscular Haemoglobin

Mean corpuscular haemoglobin values vary with the size of the erythrocyte hence values in infancy and childhood are considerably less than in adults, where it varies from a range of 23 to 30pg in childhood to values of 26 to 32pg in adults. It is a very useful index for reflecting iron deficient anaemias and also hereditary spherocytosis. The ratio of haemoglobin content to cell size gives perhaps a more reliable indication. It is interesting to notice that male values are slightly higher than the corresponding values for females.

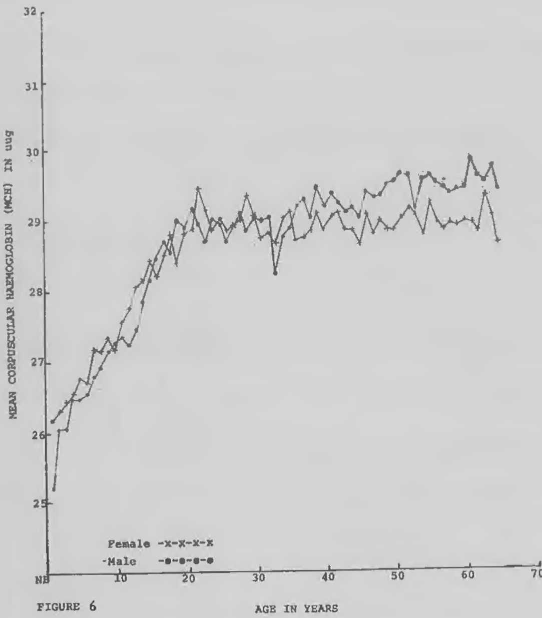


FIGURE 6

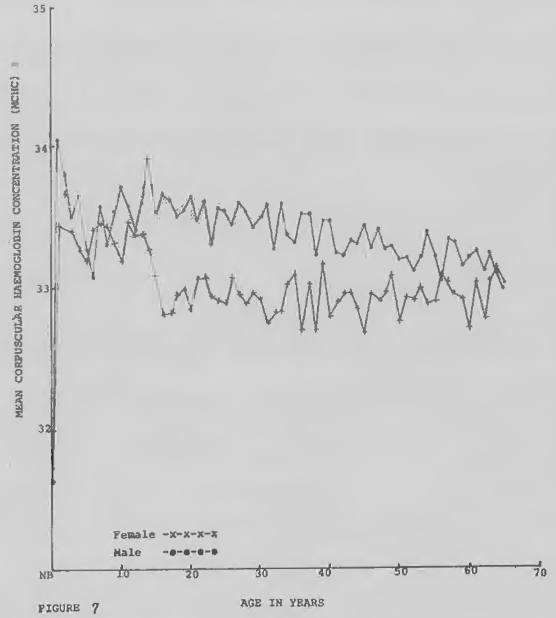


FIGURE 7

TABLE V1

MEAN CORPUSCULAR HAEMOGLOBIN

Age Group	MALES			FEMALES		
	No in Group	Mean ± 2 S.D.	Mean	No in Group	Mean ± 2 S.D.	Mean
Cord Blood	105	31.3 - 38.4	34.9	96	31.8 - 38.5	35.1
1 - 5	463	22.8 - 29.6	26.2	385	23.3 - 29.8	26.5
6 - 10	522	24.1 - 29.7	26.9	470	24.3 - 29.9	27.1
11 - 15	488	24.9 - 30.2	27.6	484	25.2 - 30.8	28.0
16 - 20	455	26.2 - 31.2	28.7	496	25.5 - 31.6	28.6
21 - 25	499	26.3 - 31.6	29.0	559	25.9 - 32.2	29.0
26 - 30	441	26.1 - 31.7	28.9	498	25.9 - 32.2	29.0
31 - 35	450	26.2 - 32.7	29.0	528	25.4 - 32.3	28.9
36 - 40	431	26.6 - 31.9	29.3	476	25.5 - 32.1	28.8
41 - 45	444	26.4 - 32.0	29.2	488	25.8 - 32.1	28.9
46 - 50	470	26.6 - 32.2	29.4	462	25.9 - 31.9	28.9
51 - 55	343	26.4 - 32.6	29.5	473	26.2 - 32.0	29.1
56 - 60	402	26.6 - 32.3	29.5	398	26.0 - 31.9	28.9
61 - 65	319	26.7 - 32.6	29.6	368	26.1 - 31.8	28.9
66 - 70	240	26.6 - 32.3	29.5	318	26.0 - 32.0	29.0

Mean Cell Haemoglobin Concentration

The results show normal values of 31 to 36 percent in children and 31 to 35 percent in adults. As with the mean cell haemoglobin, male values are again slightly higher than the corresponding values for females.

TABLEV11

MEAN CORPUSCULAR CONCENTRATION

Age Group	MALES			FEMALES		
	No in Group	Mean ± 2 S.D.	Mean	No in Group	Mean ± 2 S.D.	Mean
Cord Blood	105	29.4 - 34.1	31.7	99	29.0 - 34.2	31.6
1 - 5	463	30.9 - 36.2	33.6	385	31.0 - 35.8	33.4
6 - 10	522	31.4 - 35.6	33.5	470	31.5 - 35.3	33.4
11 - 15	488	31.6 - 35.6	33.6	484	31.4 - 35.2	33.3
16 - 20	455	31.7 - 35.5	33.6	496	31.1 - 34.7	32.9
21 - 25	499	31.6 - 35.5	33.5	561	31.2 - 34.8	33.0
26 - 30	441	31.6 - 35.4	33.5	498	31.1 - 34.8	33.0
31 - 35	450	31.4 - 35.5	33.4	528	31.0 - 34.7	32.9
36 - 40	431	31.5 - 35.3	33.4	476	31.0 - 34.7	32.9
41 - 45	444	31.4 - 35.2	33.3	488	31.0 - 34.7	32.9
46 - 50	470	31.3 - 35.3	33.3	462	30.9 - 35.0	32.9
51 - 55	343	31.3 - 35.2	33.2	473	30.9 - 34.9	32.9
56 - 60	402	31.2 - 35.2	33.2	398	31.0 - 34.9	32.9
61 - 65	319	31.1 - 35.2	33.1	368	31.0 - 34.9	33.0
66 - 70	240	31.1 - 35.2	33.1	318	30.[illegible] - 34.9	32.9

Conclusion

The results shown in this paper show the obvious need for laboratories to establish their own normal values which in turn will depend largely on the population tested and the methods used.

Acknowledgments

The author would like to thank the computer staff for their valued assistance in punching up results and processing the information, and also Miss Mary Oliver for typing this article.

REFERENCE

1. Practical Haematology 4th Edition by J. V. Dacie and S. M. Lewis.

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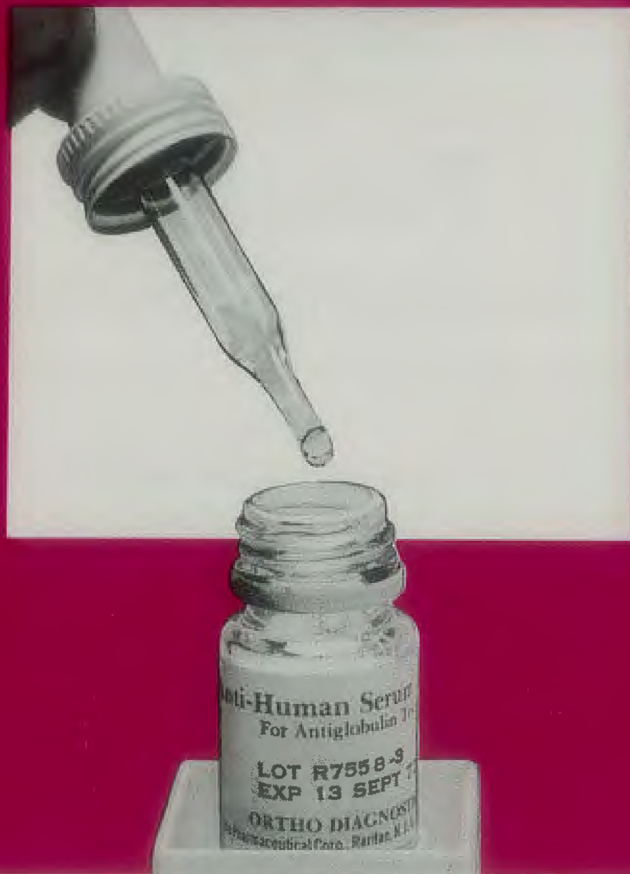

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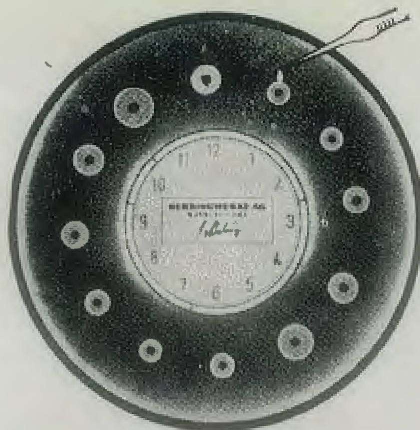

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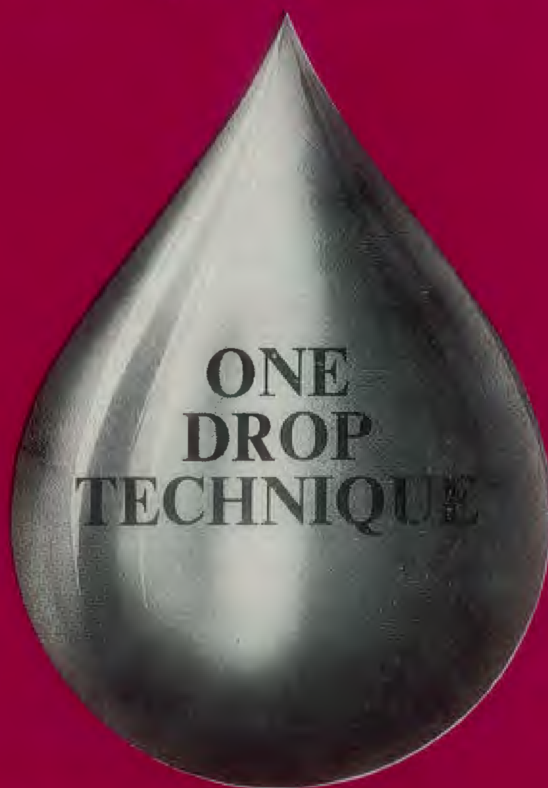

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A Micro Immunoassay Method for Measuring Luteinising Hormone and Low Levels of Human Chorionic Gonadotrophin

E. J. McKay and Barbara A. Christie

Immunology Department, Hamilton Medical Laboratory, Hamilton

Received for publication, February 1974

Summary

Minor modifications to the Luteonosticon (Organon Laboratories) Immunoassay procedure for measuring luteinising hormone (LH) and low levels of human chorionic gonadotrophin (HCG) have been made allowing a relatively simple four-hour laboratory test to be performed. A comparison with 30 urines and two commercial preparations of HCG with varying LH and HCG concentrations is made. Stability and reproducibility of reagents is also analysed.

Introduction

The biological and immunochemical assay of human chorionic gonadotrophin and luteinising hormone levels have proved to have diagnostic importance in evaluating various obstetric problems[1, 3, 4].

As early as 1926 Zondek and others showed that the urine of pregnant women possessed strong gonadotrophic action. The hypophysis secretes gonadotrophins, some with follicle ripening and others with luteinising properties. On this basis two hormones are distinguished, i.e., follicle stimulating hormone (FSH) and luteinising hormone (LH).

The placenta secretes large amounts of human chorionic gonadotrophins (HCG) which cannot be distinguished biologically from LH. After complete abortion HCG production falls rapidly; extrauterine (ectopic) pregnancy is of particular interest for HCG excretion is greatly reduced. Two pathological conditions where increased HCG excretion is found are hydatidform mole and chorionic epithelioma. Quantitative HCG levels are of diagnostic importance because these two diseases generally exhibit abnormally high levels of HCG.

Sterility in females can be due to an-ovulation so the determination of the LH peak during menstruation can be of diagnostic importance, particularly during treatment with chemical ovulation inducers[2]. In males where there is apparent dysfunction of the testes, it is important to ascertain whether gonadotrophic excretion is above or below normal levels and so distinguish true testicular damage from hypophysial damage[5].

The therapeutic management of patients with a molar pregnancy or epithelioma—involving evacuation of the uterus and use of cytostatic drugs—is considered mandatory by some authorities for as long as two years. LH excretion greater than normal may indicate a relapse especially where pregnancy has been excluded on clinical grounds. Investigation of ectopic pregnancies by LH assay techniques can be important where HCG excretion is too low to be detected by normal pregnancy tests. When HCG excretion falls below a certain level the prognosis for pregnancy continuance is unfavourable; i.e., abortion is likely to occur. Low HCG levels coincide with deviating placental tissue structure.

As normal pregnancy tests usually measure 750IU/litre of HCG or more, a rapid assay system for measuring low levels of these hormones is needed. Specific bioassays for LH and low levels of HCG are associated with various disadvantages[4]. Time-consuming extraction methods and subsequent concentration of active substances are feasible only in large and specialised laboratories . More specific and sensitive methods have been met by immunochemical analysis. Radioimmunoassay methods are still confined to specialised laboratories. Organon Laboratories have developed a haemagglutination inhibition method (HAI) for the detection of LH and low levels of HCG (Luteonosticon)[4, 6, 7].

This method has the advantage of conventional HAI systems, i.e., results obtainable within one working day, test procedure may be performed by a routine laboratory familiar

with conventional tube pregnancy tests, no expensive apparatus is required and results are easily interpreted. Nevertheless, this method is still tedious and expensive to perform. Because of these two main disadvantages it was felt a modification utilising existing micro techniques could be tried.

Materials and Methods

Microtiter Apparatus (Cooke Engineering Co.) .

1. 50μl pipette droppers;
2. Lucite "U" plates (permanent type);
3. Luteonosticon immunoassay kit. Reagents from this kit are reconstituted according to the manufacturer's (Organon Laboratories) instructions.

Reagents include: (a) Antiserum to HCG (lypholised); (b) Erythrocytes coated with HCG (lypholised); (c) Diluent.

Method Comparison

MACRO

Antiserum	Urine
0.5ml	6.0ml

Incubate 2-4hrs at RT
Add 0.5ml erythrocytes
Incubate 0-60mins at RT
Centrifuge 5-10 mins 2000 rpm
Discard supernatant
Wash erythrocytes (5ml buffer)
Centrifuge 5-10mins 2000rpm
Resuspend in 0.4ml buffer
Pour suspension into supplied tube
Read settling pattern (2 hours)

Agglutination inhibition	Agglutination
POSITIVE	NEGATIVE

Time required—7½ hours

MICRO

Antiserum	Urine
50μl	0.6ml

Incubate 2hrs at RT
Add 100μl erythrocytes
Incubate 30mins RT
Centrifuge 3400rpm 2mins
Discard supernatant
Wash erythrocytes (0.5ml buffer)
Centrifuge 3400rpm (2mins)
Resuspend in 50μl buffer
Drop suspension into microtiter plate
Read pattern after 1½-2 hours

Agglutination inhibition	Agglutination
POSITIVE	NEGATIVE

Time—4 hours

Quantitative Procedure

Tube no.	1	2	3	4	5		6
Antiserum	1*	1	1	1	1	Calculation of	— (Control)
Diluent	—	6*	9	10	11	LH Concentration	1
Urine	12*	6	3	2	1	(IU/L)	12
Cell	—	—	—	—	—	<25	+
Settling	+	—	—	—	—	25	+
Pattern	+	+	—	—	—	50	+
	+	+	+	—	—	100	+
	+	+	+	+	—	200	+
	+	+	+	+	+	>400	+

* N.B. 1 volume = 50μl

The highest dilution of urine to give a clear ring formation (i.e. complete haemagglutination inhibition) is taken as the end point. For further interpretation refer to Figure 3.

Results

A blind trial was performed analysing 30 urine samples with various concentrations of HCG/LH; as can be shown in Figure 1 these results correlated well for no discrepancies were demonstrated.

Two commercial preparations of HCG were also analysed (Pregnyl—Organon Laboratories and CG-2—Sigma Chemicals). Varying concentrations of these preparations were diluted in both distilled water and a urine sample which demonstrated less than 10 IU/litre of HCG. Good correlation was achieved and may also be seen in Figure 1.

Reproducibility studies of eight replicate urine samples with differing HCG/LH concentrations showed little or no variation in hormone levels. Ten urines having different HCG/LH levels were re-examined using reagents reconstituted for 5, 10, 15, and 20 days. These reagents showed no loss of reactivity until 15-20 days. This is shown in Figure 2.

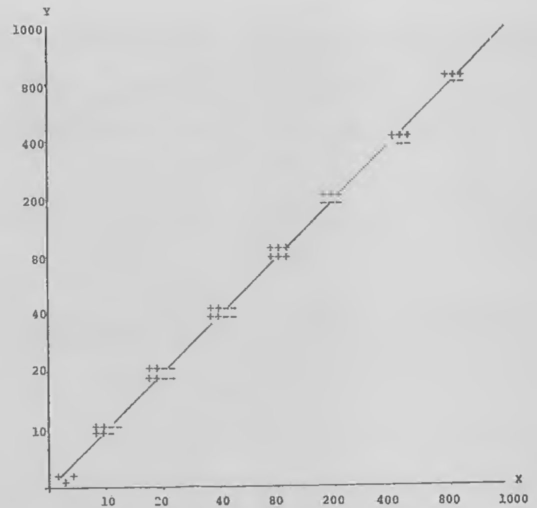

Figure 1. Comparison of varying HCG levels by both methods

X Luteonosticon
Y Micro method
+ = Urine samples
– = Commercial HCG preparations

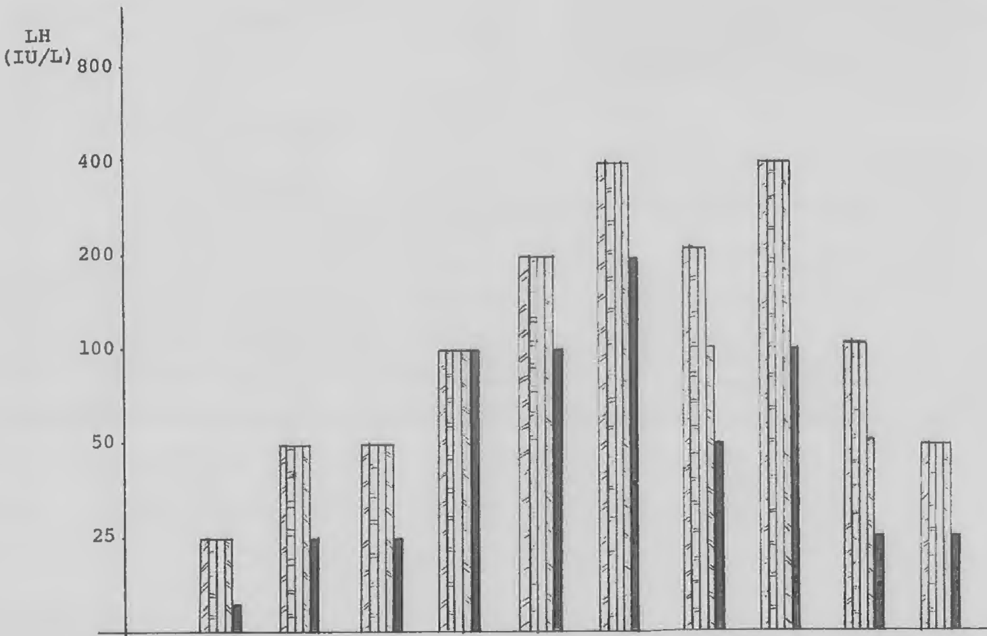

Figure 2. Comparison of 100 Urine Samples using Reagents up to 20 days after reconstitution.

Day 1 Day 10 Day 20
Day 5 Day 15

Footnote: *Erratum: Caption should read 10 urines.*

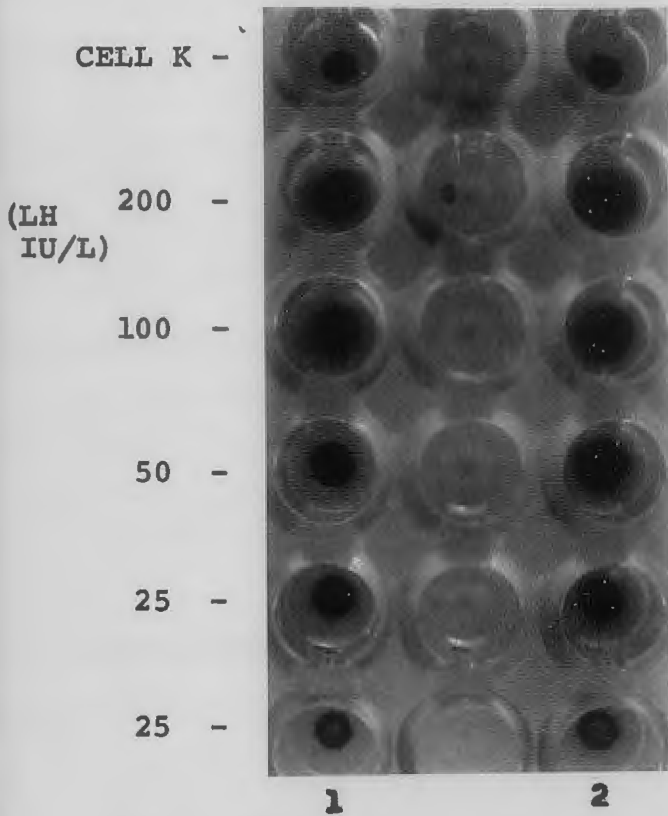

FIGURE 3.—Typical Quantitative results by Micro HAI procedure. No. 1. Has an LH level of 100 IU/L. No. 2. Has an LH level of 25 IU/L.

Discussion

These minor modifications to Luteonosticon enables a micro haemagglutination inhibition test to be performed within a relatively short time (i.e., four hours).

Routine heating of urine samples for 5-10 minutes in a water bath which has been brought to the boil, has eliminated the occasional test sample from exhibiting an abnormal settling pattern. Microtiter U shaped lucite plates facilitate easier interpretation of end points when quantitating test samples (see Figure 3). The eight-fold increase in economy, relative stability of the reconstituted reagents (10 days), and simplicity of the procedure makes this method eminently suitable for prolonged studies such as monitoring molar pregnancies after treatment and ovulation studies in infertile females.

This method has been used in this Laboratory for more than two years and has proved a useful laboratory aid in obstetric problems.

Acknowledgments

We wish to thank the pathologists of Hamilton Medical Laboratory for allowing us to present this material, particularly Dr B. J. Linehan for his advice and criticism. We also thank Mrs H. M. Davison and Miss J. E. Allen for their technical assistance.

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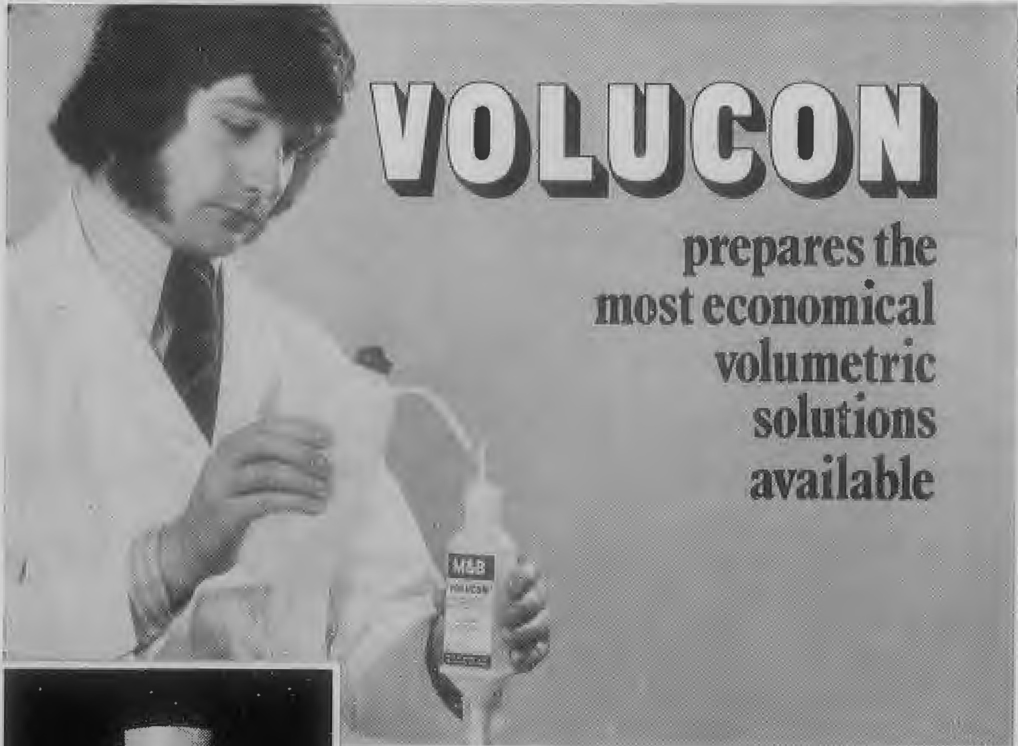

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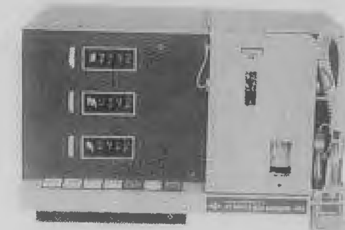

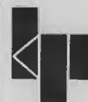

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An Assessment of the Pathotec Rapid I-D System

G. L. Cameron, ANZIMLT

Department of Microbiology, Auckland Hospital

Received for publication, March 1974

Summary

The Pathotec Rapid I-D System was used in parallel with standard tubed media to identify 25 gram-negative rods. In addition, one isolate of Klebsiella was tested 12 times. The tests used, the methodology of the tests, the results and discrepancies obtained are discussed.

It is concluded that Pathotec strips are suitably reliable in the identification of most members of the family Enterobacteriaceae to genus level.

Introduction

Numbers of commercially produced kits for the identification of the members of the family Enterobacteriaceae are obtainable. They differ in their methods, presentation and reliability. The Pathotec Rapid I-D System, developed by the Warner-Lambert Company, consists of a series of paper strips impregnated with substrates, indicators and/or reagents. They are designed to give readable results after four hours' incubation with a standardised saline suspension of organisms. The tests included in this kit are cytochrome oxidase, nitrate reduction, phenylalanine deamination, urease, indole. H_2S and lysine decarboxylase production, Voges-Proskauer reaction, malonate utilisation, aesculin hydrolysis. It was decided to test this new set against a similar set of conventional tubed macro methods.

Methods

Only the methods used with the strips will be described.

All the macro-tube methods used are standard methods as set out by Cowan and Steele[1] and were read after 18 hours' incubation at 36°C

The exact composition of each strip varies, but in general takes the form of an absorbent paper strip 5mm wide by 80mm long. One end is colour coded as to test, and the other is impregnated for a length of 15mm with the substrate, together with the indicator where applicable. Tests requiring additional reagents, have that reagent impregnated into a similar area of the strip 2cm above the substrate, and separated from it by a water impervious area to prevent diffusion and resultant premature mixing.

A suspension of the organism to be tested is prepared in physiological saline, and 0.3ml is dispensed into as many small test tubes as is required. Strips are placed in the tubes so that the substrate is immersed, the tubes are capped, and the whole is incubated at 36-37°C for four hours.

Following incubation the tests are read, either by observing the colour of the now dissolved indicator, as in the case of the urease, and malonate strips; by tipping the tube to wet the reagent impregnated area with the suspension, and observing colour development in the strip, as in the phenylalanine, indole, and nitrate reduction strips; or as in the case of the V-P, H_2S, oxidase and aesculin strips, by a special method applicable to the test.

The methods adopted for the strips are standard ones, modified to suit the system.

Nitrate reduction

The test zone is impregnated with nitrate, and the reagent zone with sulphanilic acid and alpha-naphthylamine.

When the test is positive the normal pink colour develops in the reagent zone after the tube is tipped to wet that zone. The limited incubation time should ensure that sufficient nitrite remains to react where organisms which reduce this to nitrogen are being investigated. Consequently there is no need to check negative reactions with zinc dust[3]. The strips are supplied in a brown glass vial, to prevent deterioration of the reagents on exposure to light. The colour reaction was always rapid and easy to read.

Urease production

Urease and phenolphthalein are both impregnated into the substrate zone. The bright pink colour which develops on the splitting of urea was readily observed.

Indole production

The substrate zone contains tryptophane. The reagent zone which is impregnated with

Ehrlich's reagent is wetted after incubation, producing an immediate and characteristic colour change in the presence of indole. In most cases the colour was intense, although some organisms gave a weaker response.

Lysine decarboxylase

Both lysine and indicator are impregnated into the substrate zone and the positive colour change to purple is observed after incubation. Some positive reactions were rather weak, but were enhanced on further incubation. This test was one of the more difficult ones to interpret.

Phenylalanine deamination

The substrate zone contains phenylalanine, and the reagent zone, ferric chloride. When wetted by tipping the tube after incubation, the reagent zone turns green immediately when phenyl-pyruvic acid is present.

Malonate utilisation

Both substrate and indicator are incorporated in the substrate zone. A positive reaction is indicated by the blue green colour developed in the suspension.

Hydrogen sulphide production

A detection zone of lead acetate is placed so as to be located 4cm above the surface of the suspension. The presence of volatile H_2S at that level is detected by the blackening of the strip. In order to produce a test of suitable sensitivity, an " absorbent zone " is interposed between the substrate zone and the detection zone. Just what this " absorbent zone " contains is not made clear, but as this zone turns black, it must be assumed that it also contains some lead acetate.

Voges-Proskauer reaction

Buffered glucose substrate in the substrate zone and alpha-naphthyl in the reagent zone are separated by an interposed waterproof zone. After incubation two drops of 400 percent of KOH is added to the suspension, mixed, and the tube tipped to wet the reagent zone. The typical pink colour develops after from 10-20 minutes. Colour development was occasionally rather pale.

Aesculin hydrolysis

Aesculin, and a ferric ion containing substance are impregnated into the substrate zone. A single colony of the test organism is rubbed on to the zone, which is then moistened with saline, the strip is incubated in a tube. The production of aesculetin by the hydrolysis of aesculin produces a black colour due to interaction with the ferric ions.

Cytochrome oxidase

Two similar zones on each strip are impregnated with p-phenylenediamine and alpha-naphthol. A single colony is rubbed on to one zone and a positive control organism is rubbed on to the other. The typical purple-black colour develops rapidly.

Results

Twenty-five organisms were tested in parallel with the strips and by routine methods. They were *Pseudomonas aeruginosa, Citrobacter freundii* Providence sp, *Proteus vulgaris,* Enterobacter sp, *Shigella dysenteriae,* Serratia sp. *Salmonella typhimurium, Yersinia enterocolitica, Yersinia pseudotuberculosis, Pasteurella multocida, Enterobacter agglomerans, Escherichia coli* × 6, Klebsiella spp × 5. In addition, one isolate of Klebsiella was tested 12 times by the strips. Of the total 370 separate reactions performed, 15 discrepant results were recorded (Table I). No discrepancies were found in the results of the repeated testing of the Klebsiella sp, which gave consistent and expected reactions (Table II).

TABLE I

		Oxidase		Nitrate		Phenylalanine		Urease		Indole		H2S		Lysine		V-P		Malonate		Aesculin	
		P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C
Ps. aeruginosa	1	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Cit freundii	2	−	−	+	+	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−
Providence sp	3	−	−	+	+	+	+	−	−	[−]	+	−	−	−	−	−	−	−	−	+	+
Prot. vulgaris	4	−	−	[−]	+	+	+	+	[−]	+	+	+	+	−	−	−	−	−	−	−	−
Enterobacter sp	5	−	−	+	+	−	−	−	−	−	−	−	−	−	−	+	+	[−]	+	+	+
Prot. mirabilis	6	−	−	+	+	+	+	+	+	−	−	+	+	−	−	+	+	−	−	−	−
E. coli	7	−	−	+	+	−	−	−	−	+	+	−	−	+	[−]	−	−	−	−	−	−
Sh. dysenteriae	8	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Serr. marcescens	9	−	−	+	+	−	−	−	−	−	−	−	−	−	−	+	+	−	−	+	+
E. coli	10	−	−	+	+	−	−	−	−	+	+	−	−	+	[−]	−	−	−	−	−	−
Klebsiella sp	11	−	−	+	+	−	−	+	+	−	−	−	−	−	−	+	+	+	+	+	+
S. typhimurium	12	−	−	+	+	−	−	−	−	−	−	+	+	+	[−]	−	−	−	−	−	−
E. coli	13	−	−	+	+	−	−	−	−	+	+	−	−	+	+	−	−	−	−	−	−
E coli	14	−	−	+	+	−	−	−	−	+	+	−	−	+	+	−	−	−	−	−	−
Klebsiella sp.	15	−	−	+	+	−	−	[−]	+	−	−	−	−	+	+	+	+	+	+	−	−
Klebsiella sp.	16	−	−	+	+	−	−	[−]	+	−	−	−	−	[−]	+	+	+	−	−	+	+
E. coli	17	−	−	+	+	−	−	−	−	+	+	−	−	+	+	−	−	−	−	−	−
E. coli	18	−	−	+	+	−	−	−	−	+	+	[+w]	−	−	−	−	−	−	−	−	−
Cit. fruendii	19	−	−	+	+	−	−	+	+	−	−	+	+	−	−	−	−	+	+	−	−
Klebsiella sp.	20	−	−	+	+	−	−	[−]	+	−	−	−	−	+	+	+	+	+	+	+	+
Klebsiella sp.	21	−	−	+	+	+	+	[−]	+	−	−	−	−	+	+	+	[−]	+	+	+	+
Past. multocida	22	+	+	+	+	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−
Yers. pseudotuberculosis	23	−	−	+	+	−	−	+	+	−	−	[+]	−	−	−	−	−	−	−	−	−
Yers. enterocolitica	24	−	−	+	+	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−
Ent. agglomerans	25	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

P = PathoTec strips C = conventional methods w = weak

Results of comparative tests. Symbols enclosed in boxes are discrepant.

TABLE II

	OXIDASE	NITRATE	PHENYLALANINE	UREASE	INDOLE	H_2S	LYSINE	V-P	MALONATE	AESCULIN
Klebsiella sp	−	+	−	+	−	−	+	+	+	+

Reactions of Klebsiella tested 12 times without discrepancy.

Discussion

The problems of identifying gram-negative rods in general, and members of the family Enterobacteriaceae in particular, are many. The range of variation displayed within a given species, makes it difficult to place a significant number of clinical isolates. Many schemes have been devised, all of which satisfy the demands of the devisor, and numbers of commercially prepared kits have appeared over the years.

In 1954 Warner-Lambert produced Pathotec strips; seven tests which it was claimed would give an identification in four to five hours. The tests were indole, phenylalanine deaminase, oxidase, urease and lysine decarboxylase production, citrate utilisation, and the Voges-Proskauer reaction. More recently the set has been expanded to 10 strip tests with the inclusion of tests for nitrate reduction, malonate utilisation, aesculin hydrolysis, and hydrogen sulphide production, and the deletion of the citrate utilisation. The strips themselves have been improved and some of the methods changed to give more reliable results.

Results obtained in this survey show a 98 percent correlation between the conventional methods and the Pathotec strips. An examination of the fifteen discrepancies show that 10 were due to a deviation from the expected result with the Pathotec strips, and five with the conventional methods. Of the 10 strip discrepancies, eight were false negative reactions and two were false positive. Four of the eight false negatives were due to the failure to detect urease activity of Klebsiella spp. As this activity can be delayed in these organisms, further incubation would have probably corrected the anomaly. The most serious error was the failure to detect nitrate reduction in a *Pr vulgaris* strain (org. 4). Failure to detect this basic reaction was potentially misleading. The false positive reactions were the detection of H_2S production by one *E. coli* isolate and *Yersinia pseudotuberculosis*. The assessment of H_2S production depends upon the sensitivity of the test employed. Lead acetate is an extremely sensitive indicator, and is not regarded by some authorities as the method of choice for Enterobacteriaceae. In fact Edwards and Ewing[2] state that it should not be used in this context.

The makers have attempted to adjust the sensitivity of the test by the inclusion of an absorption zone. However, the H_2S producing capacity of Enterobacteriaceae is so variable, that it is almost inevitable that some result such as this is going to occur from time to time. The level of sensitivity of the test is difficult to establish, and probably impossible to maintain with precision. The five failures of the standard media included three false lysine decarboxylase tests, one each of urease, V-P, and H_2S detection. These anomalies were perhaps more serious than those of the strip test because three of them are key reactions (lysine decarboxylase). The distribution of these failures would point to faulty medium, as the tests were done in two batches (organisms 1-12 and 13-21).

Conclusion

The tests included in the Pathotec Rapid I-D System, provide sufficient information to identify most Enterobacteriaceae isolates to genus level and many to species level. The major criticism is the inability of the kit to distinguish between Klebsiella and Enterobacter species without resorting to a motility test. An answer to the problem, would seem to lie in the inclusion of a test for ornithine decarboxylase. Further, the deletion of the citrate test from the new kit is to my mind a pity. This key reaction would seem of greater value than aesculin hydrolysis in this context.

While organisms outside the family Enterobacteriaceae are not specifically catered for, sufficient information is gained about such isolates to promote relevant second stage testing.

From a practical point of view the system has a few pitfalls. The pipetting of 0.3ml aliquots of suspension, if not done with some pipetting aid could be a hazard, particularly where large numbers of isolates are being tested.

Another potential hazard arises out of the form of the tests. Small strips of paper with coloured ends do not equate in most technologists' minds with live cultures, and unless carefully educated in their use staff could

create dangerous situations due to careless handling.

The cost of the system compares very favourably, test for test, with standard methods. This is particularly so in laboratories preparing or buying large quantities of media.

In addition, the separate packaging of individual tests makes the system very flexible as the choice of tests is then left to the bacteriologist involved.

Taking all the above factors into consideration, the Pathotec Rapid I-D System would appear to be a reasonable and reliable alternative to the routine use of conventional tubed media.

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From Serology to Immunology— History of a Laboratory

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Introduction

Interest has been shown recently in early laboratory history (Buxton, 1973)[4]. Notes on a specialised department may be of interest as the Auckland Hospital has pioneered both a separate Department of Serology, and its extension into the wider field of immunology.

Early Work

Initially serology was syphilis serology only. In Auckland in the 1930's F. L. Armitage, the bacteriologist, performed Wassermanns. At the beginning of World War II after the death of Armitage, Douglas Whillans became bacteriologist who then first shared perfomance of syphilis serology with the pathologist, Dr Selwyn Hills (WR and Kahn) later running it on his own, although still responsible to the pathologist. Douglas Whillans set the pattern for the Department of Serology, which has remained essentially the same up till recently, that non-medical staff were in control. He was helped by working together with A. A. Rosenberg at the USA 39 General Hospital, stationed in Auckland. Rosenberg, who was one of the authors developing the VDRL test, was experienced in a variety of flocculation tests and the preparation of Kahn reagents, haemolysins, etc. Important original work was done by Whillans developing a 50 percent haemolysis method, devising a photelometer to measure the unhaemolysed cells, and applying accurate statistical methods and calculations (Whillans, 1950)[18].

The idea of extending both microbiological serology and nonbacterial immunological work had been conceived by Dr Hills, in the early fifties, and Douglas Whillans set about to put this into practice. One of the first such tests developed was the Rose-Waaler test, on the request of Rotorua Queen Elizabeth Hospital, including the inhibitions, a tricky procedure. This was immunological work already. When I joined the department in the middle fifties we had time to expand further. A modified Rose-Waaler was developed and published (Whillans *et al.*, 1958)[19]. Previous work on protein fractions (Fischman, 1954, 1957) gave me the idea, on which this was based. Most New Zealand laboratories have sent their sera for this test to us for many years.

Towards the end of the fifties Douglas Whillans contributed considerably to the development of serum complement estimations, but after this he had to devote all his time and energy to the job of principal technologist, leaving—somewhat reluctantly—control and further development to me.

The Personal Touch

I have done serology first in a laboratory in Hungary. Widals were performed with live bacterial suspensions. My first activity included getting this live suspension on my hand. Reading of WR's and Gono slides (no culture then) was a sacred performance, the prerogative of the Director of Pathology. Occasionally the wards complained that 70-year-old females

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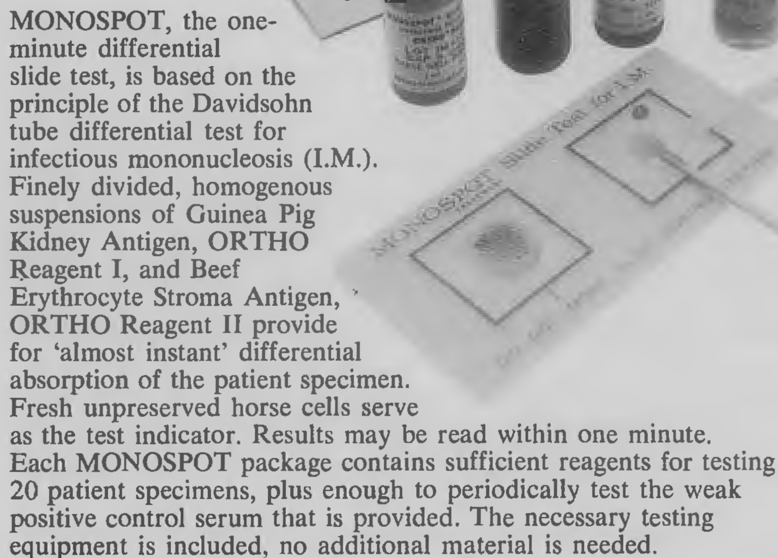

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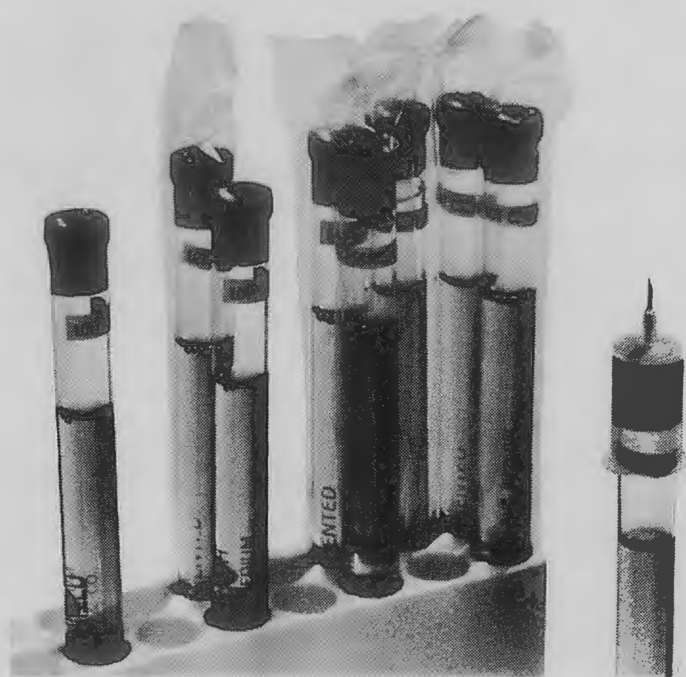

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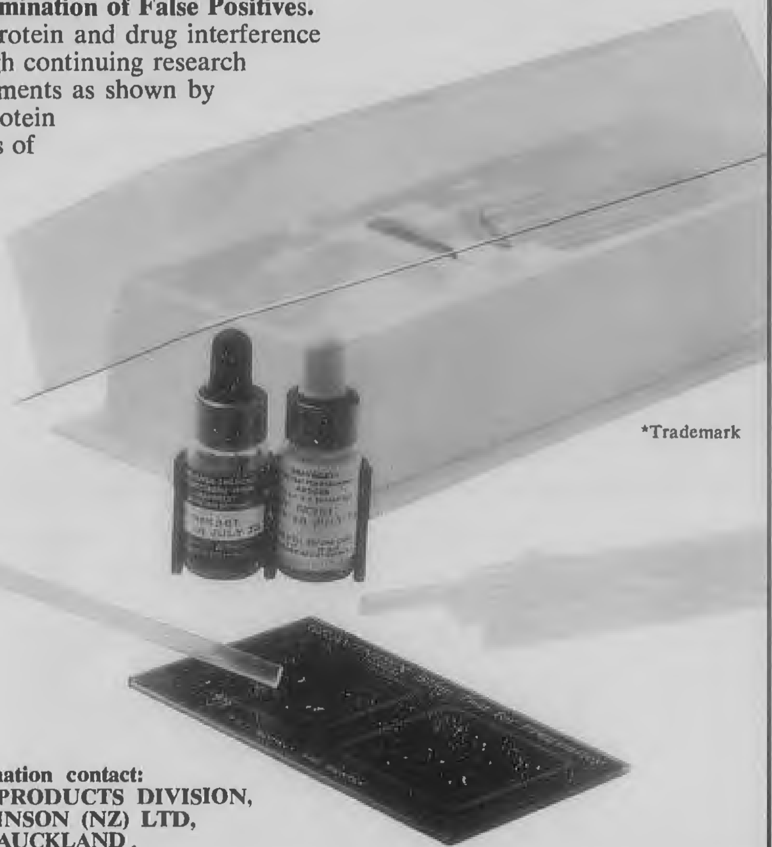

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were reported to have the gram-negative diplococcus. Part of the skill was to prepare all reagents, haemolysins, antigens, obtain complement by heart puncture of guinea pigs, to obtain sheep cells by venepuncturing the one and only sheep kept in the hospital grounds, and lured to the laboratory with carrots (and stick).

My initiation in the Auckland Hospital was not without the occasional obstacle. Thus when I was assigned night duty roster with one of the female staff, she refused to share the roster with me, having some odd ideas about foreigners. The rest of the staff offered the consolation that I should consider this as a compliment rather than an affront. One of the senior staff had given me official advice how I should move, bend, etc., in order not to startle anybody. *O tempora, O mores!* Who is worrying about this nowadays. In those days skirt length was strictly regulated.

A senior biochemist (strangely enough also female) wanted to prevent me from venepuncturing patients, in case they do not understand my English (today there is less bias against continental accent; more against a British Isles one).

Early Immunology

The late fifties saw the important breakthrough in autoimmune diagnostic serology; the precipitin test for thyroid antibodies. We managed to produce a good antigen, and some positive tube and plate agar diffusion reactions, in time to exhibit at the Commonwealth BMA Conference.

The next step was to prepare a calf thymus nucleoprotein antigen, a very cumbersome procedure, for the antinuclear factor complement fixation test. This was used until replaced by fluorescent microscopy.

Immunological work outside Auckland Hospital laboratory received a boost when Ken Couchman came from London in the middle sixties and started to do tissue antibody fluorescence in Palmerston North. We arranged with him that we would not develop this in the meantime but leave this for Palmerston North to do only, on a national basis. In 1965 I suggested to the 21st Birthday National Conference to add to the existing division of structure an immunology forum and was asked to chair this in Tauranga. We pointed out that it is now justified to consider immunology a separate laboratory entity. A review included a note on cancer immunology, now more in the news. The 1966 Hamilton Conference also held an immunology forum, but subsequent conference organisers did not bother any more.

The idea of separate serological departments gathered momentum in the late sixties to a number of other laboratories and was facilitated greatly by the increasing production of serological commercial reagents. Many of these may be used by less trained personnel, and some of the skills involved in the preparation of reagents was replaced by a skill, choosing between the proliferation or reagents offered by commercial firms.

Communicable Diseases

Microbiological serology has been extended in the fifties to include viral complement fixation. Influenza antibodies have been determined wholesale in the great flu epidemic (this work has been later transferred to the Virus Department). Hydatid serology has been increased by the addition of agglutination tests, when I have developed the Latex and Bentonite tests (Fischman, 1960)[9, 10].

This was then one of the first applications of the Latex technique beside RA. Since then Latex found many other uses, and predictably became a goldmine for manufacturers of reagents. I was asked once by a New Zealand firm to produce latex commercial reagents, but was not sufficiently business-minded for this.

Techniques using scolex antigens have been developed later in our laboratory (Fischman *et al.*, 1967[13], 1968[12], 1970[3]; Craig *et al.*, 1970[5]). Hydatid research gave us the opportunity to introduce several immunochemical techniques, immunoelectrophoresis, diffusion, sephadex separation, and invitations to various international conferences.

In the field of antitoxin serology, an AST and antistaphylolysin micromethod has been developed by Dawkins (1964)[6] and Mundt (1970)[17] in a continuation of this, developing an antileucocidin method, was a winner of the QTO thesis prize.

The bread and butter line of serology, syphilis or rather treponemal serology, after decades of domination by reagin tests only, plus the cumbersome TPI, changed in the early sixties with the advent of the more specific treponemal tests, first the Reiter Complement fixation, and soon after the FTA. I visited Atlanta CDC, USA, in 1962 receiving firsthand information from Dr Peacock who discovered and was in

the process of improving the FTA. We soon tried to develop this in Auckland, but were hampered for a while by poor commercial antigens (in Atlanta the fresh rabbit treponemes were used, to which we had no access), and also inferior equipment. However, eventually we succeeded. The main share of developing this and other fluorescent work was due to the work of Begg (1967[1], 1970[2]). This work and further expansion is being continued by David Bree and Robert Lynch.

In 1968 we were designated National Treponemal Reference Laboratory which entails various tasks. Some aspects of this work have been reported (1972)[14] and in the yearly reports of the Health Department which includes statistical work being done by Carol Wright.

A new simple technique using Reiter treponemal antigen has been devised in this laboratory (1964)[11]. This has the merit of being the simplest treponemal antigen test, also the only indigenous New Zealand syphilis test. However, the rise of T. pallidum tests, FTA-ABS and TPHA are reducing the use of Reiter antigen tests. On my overseas visit in 1969 I saw the micro TPHA in operation, and on return introduced it in New Zealand. This test and the RP latex was found useful when tackling the problem of syphilis and yaws differentiation (Fischman *et al.*, 1971[15], 1973[16]).

Present and Future Scope

For various reasons, some administrative, some sentimental, the department retained the name serology, and was officially changed to immunology only a year ago. This was accompanied by an administrative change of becoming independent, breaking the previous loose ties with microbiology, and adding a medical immunologist to the establishment.

With immunology now a recognised specialty and departments already established, or planned in other New Zealand centres, a note on its scope is in order. At present this is variable in different laboratories. Some of the work is scattered in various other departments (e.g., radioimmunoassays, transplant immunology), while some of the tests are not yet done anywhere. The scope can be defined in two ways. (1) the type of technique, (2) fields of pathology. As to (1) there is no doubt, that fluorescent microscopy, immunoelectrophoresis, diffusion, sephadex separation, etc., must form an integral part of the department. But it is a local political question, how much if any of this done in other departments already, should be transferred, or duplicated. An example is immunoelectrophoresis, which was first performed in Auckland, in the blood bank, mainly for research, later for semi-routine diagnosis, and is also being done now in the Department of Biochemistry. One cannot make any technique a monopoly of one department, but too much duplication is expensive. Many techniques in immunology are still essentially serological tests, but there is an increase in different types. The rôle and limitations of automation also have to be looked into.

As to the fields of pathology to be included, the symbiosis of microbiological serology, with the non-microbiological immunology worked very well in Auckland Hospital, giving wide accumulated experience to the department. Regarding microbiological serology, however, it was fairly eclectic, what has been performed by us, and what by the Department of Bacteriology. There was a vague guiding principle originally; namely tests using infectious antigens were dealt with in bacto (e.g., leptospira). More clear cut is the whole field of autoantibodies: complement and its fractions: immunoglobulins. New fields include circulating immunecomplexes, the expanding area of cell-mediated immunity relating both to lymphocytes and to neutrophils; immunotherapy including cancer surveillance, etc. In these latter fields there is not always clear division yet between research and routine. Immunology and serology have often been somewhat more research-orientated than other departments.

The scope is unlimited and offers a variety of challenges.

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Technical Communications

An Unusual Rhesus Antibody

In May 1973 a gravida 4, 23-year-old woman (Mrs De W) presented at Westown Maternity Hospital, New Plymouth. Antibody screening of the serum of Mrs De W showed the presence of Anti E which reacted by enzyme but not Coomb's technique; the blood group of Mrs De W was B R_1r. Later studies during this pregnancy showed that this antibody reacted also at 37°C in saline, but only weakly by albumin 37°C technique.

Post-delivery the following results were obtained on the serum of Mrs De W:

Anti E	*Technique*	*Titre*
	Enzyme 37°C	⩾ 512
	Indirect Coombs Technique (I.C.T.) 37°C	Nil
	Saline 4°C	8
	Saline 22°C	8
	Saline 37°C	16
	Albumin 37°C	2

Several anti-human globulin sera were used in an attempt to invalidate the above negative I.C.T. finding.

Mrs De W had had four children:

Pregnancy		*Group*
1	Stillbirth	
2	S. De W	B rr
3	L. De W	BR_1r
4	D. De W	O rr

Unfortunately blood from Mr De W is unavailable for study.

Mrs De W was transfused with four units of group O D Positive blood during her admission to hospital after her first pregnancy. Subsequent groupings of these four donors show that two are group O R_1R_1 and two are group O R_1r. Mrs De W has no other history of transfusion, or pregnancy.

Numerous examples of naturally occurring Anti E have been documented[1, 2]. It is possible that the serum of Mrs De W is of such type, although antibody stimulation by the first pregnancy cannot be excluded. However, features of this antibody such as activity at 37°C in a saline medium but not at 37°C by I.C.T. indicate a natural origin, but if so the high titre is an unusual feature.

March 1974. A. E. White FNZIMLT.

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A Test for Detection and Determination of Low Levels of HCG and LH

A new test kit for detection of low levels of human chorionic gonodotrophin (H.C.G.) and leuteinising hormone (L.H.) "Higonavis", has been announced by Mochida Pharmaceuticals Co. Ltd., Tokyo, Japan. The test is based on a haemagglutination reaction and can detect down to 5 I.U. H.C.G./L which is equivalent to 12.5 i.u. LH/L. This is close to the level of detection using radioimmunoassay. Levels above 150,000 I.U./L cause a prozone effect and give negative results.

A suitable dilution of the urine is prepared according to the sensitivity required and an aliquot of this is added to a vial of lyophilised red cells which have been reconstituted with buffered saline. The vial also acts as a reaction tube and fits into a mirror-rack for reading after two hours. The solutions required for preparing the urine dilutions and for reconstituting the lyophilised cells are supplied with the kit.

The detection of low levels of H.C.G. is important in the follow-up after removal of hydatidiform mole or chemotherapy of carcinoma, in the investigation of hypophysial

function and in the detection of ovulation.

The manufacturers have shown that the lyophilised cells are stable for at least one year at temperatures up to 45°C. The urine diluent and the reconstituting fluid are stable at room temperature but should be stored in the refrigerator after being opened. The test should be performed between 10°C and 25°C. Below 10°C sedimentation rings were fainter and above 25°C the sensitivity of the reagents was lowered. Filtration did not affect the results but clearer sedimentation rings were obtained with filtered urine. It is therefore recommended that urine should be filtered through the filter tubes provided. pH levels between 4 and 10 as well as various concentrations of sodium chloride and glucose were shown to have no effect on the reaction. The effect of various protein levels on the reaction has been studied. Levels of human serum albumin above 0.05g/L caused an increase in the sensitivity of the test. Human gamma globulin concentrations of 0.5g/L or greater inhibited the formation of a smooth mat of cells giving a rough ring in the presence of H.C.G.

The effect of various pretreatment methods recommended by Organon Laboratories for samples being tested with their high sensitivity H.C.G. test (Leuteonosticon) was evaluated for the Higonavis test. It was found that both freezing the urine and heating the urine 85°-95°C for 10 minutes resulted in considerable loss of H.C.G.

Higonavis is well presented and easy to use. Further testing will be required to establish the manufacturer's claims but it would appear that the estimation of H.C.G. levels in the importance range below 1000-2000 I.U./L detected by standard pregnancy tests[1] will be easily handled by any laboratory interested in doing so. It could well provide a simple and rapid alternative to radioimmunoassay.

M. Killip,
National Women's Hospital,
Auckland.

December, 1973.

REFERENCE

1. Bagshaw, K. D., Wilson, H., Dublon, P., Smith, A., Baldwin, M., and Kardana, A. (1973). *J. Obstet. and Gynaec. Brit. Comm.* **80.** 461.

A Case of Congestive Heart Failure

In March 1974, Mr E.E.F., age 86, was admitted to Wakari Hospital, Dunedin, for radiotherapy to basal cell carcinomata on his face. He also had heart failure which was being controlled by diuretic therapy.

Signs of congestive heart failure and basal cell lesions were the only findings on admission. Routine laboratory investigations revealed an elevated blood urea (120 mg/dl) and creatinine (2.8 mg/dl) which were thought to be produced by his diuretic therapy.

Liver function tests were performed by chance and revealed an elevated alkaline phosphatase of 2900 IU/litre (N 30-90 IU/litre). Proteins, bilirubin and transaminase were normal.

We do not usually do liver function tests by chance. Owing to a fault in the photocopy machine, a white line obscured one digit of the specimen number and liver function tests were performed as a bonus.

A further specimen was requested to confirm these results and to investigate the origin of the elevated alkaline phosphatase which was 2900 IU/litre.

Heat stability tests showed that after heating the serum for 15 min at 55°C, less than 25 percent of the alkaline phosphatase remained, indicating bone origin. No alkaline phosphatase remained after the serum was heated for 5 min at 65°C. This eliminated Regan-type alkaline phosphatase.

Gamma-glutamyl transpeptidase 57 mμ/ml (N up to 50 mμ/ml). This is a useful test to differentiate between a raised alkaline phosphatase of bone origin and one of liver origin. The gamma-glutamyl transpeptidase is generally raised when the liver is involved and is normal when bone is involved.

Other significant results were:

Serum calcium	9.0 mg/dl (N. 8.5-10.3 mg/dl)
Serum phosphate	2.8 mg/dl (N. 2.5-4.5 mg/dl)
Total acid phosphatase	5.9 units/dl (N. 0-4 units/dl)
Formal stable	4.2 units/dl (N .0-3 units/dl)
Tartrate labile	1.0 units/dl (N. 0-0.8 units/dl)

The cause of Mr E.E.F.'s congestive heart failure became apparent and total body X-rays were taken. His diagnosis was a well-established state of Paget's Disease. The patient showed no clinical signs of this condition.

Paget's Disease of bone, also known as osteitis deformans, is among the most common of the chronic skeletal diseases. It is an acquired disorder of unknown aetiology characterised by destruction and formation of bone. The newly formed bone however is expanded, soft and poorly mineralised osteoid tissue. As a result, the bone is unusually light and almost has the consistency of dry bread (Robbins, 1969)[2].

The incidence of Paget's Disease is difficult to determine since it is often asymptomatic and is usually detected while investigating other disorders.

The disease occurs rarely under the age of 35 years and is much more common in males than females.

As might be expected from the marked osteoblastic activity the serum alkaline phosphatase is characteristically high, often higher than any other bone disorder.

The increased bone porosity leads to the formation of large arterio-venous communications within the bone and these in turn can lead to congestive heart failure. Betro *et al.* (1973)[1] in a study of the usefulness of gamma-glutamyl transpeptidase found that the incidence of increased gamma-glutamyl transpeptidase in people with congestive heart failure was 61 percent. In patients with Paget's Disease, the increase in alkaline phosphatase was out of proportion to the slight increase in gamma-glutamyl transpeptidase.

The concentration of calcium ions is usually maintained at normal levels by reutilisation of organic phosphate ions and a feedback control of parathyroid hormone secretion. The concentration of phosphate ions is normal or slightly elevated.

Small increases in acid phosphatase are sometimes seen. The increase is generally in non-prostatic acid phosphatase.

Thus, Mr E.E.F. was a case of congestive heart failure caused by Paget's Disease. He presented asymptomatically and the key to his diagnosis was through a chance liver function test revealing an elevated alkaline phosphatase.

Full laboratory screening is thought by some to be a waste of time and money, but this is one case where laboratory screening proved of value.

Lexie R. Friend, ANZIMLT,
Diagnostic Laboratories,
Dunedin Hospital.

May 1974
From a paper read at the South Island Seminar, Timaru.

REFERENCES

1. Betro, M. G., Oon, R. C. S., and Edwards, J. B. (1973). *Amer. J. clin Pathol.* **60**, 672.
2. Robbins, S. L. (1969). *Pathology,* 3rd Edition. Chap. 31, 1336. W. B. Saunders Co.

A Stromatolysing and Cyanide Reagent for use with the Coulter Counter Model S

The Coulter Model S cell counter is now used in many of the larger haematology departments throughout New Zealand. In it, the measurement of haemoglobin and nucleated blood cell count depends on the use of a solution which will stromatolyse the erythrocytes completely and form a haemoglobin compound presumably cyanmethaemoglobin, within 20 seconds. The haemoglobin compound is then read spectrophotometrically and the nucleated blood cell count is performed according to the Coulter principle.

The Lyse-S reagent (supplied by Coulter Electronics, Aust.) performs this satisfactorily but is very expensive. So recently, when a suitable substitute solution was described by Skinnider and Musclow[1]. a comparison of solutions was made in our laboratory. The original formula was modified slightly due to the availability of chemicals i.e., cetrimide powder was substituted for ethylhexadecyldimethyl ammonium bromide and Nonidet P40 for Brij-35.

The results were an entirely satisfactory reagent, and scattergraph comparisons of the two reagents with haemoglobin and white cell count values were similar to that found by Skinnider and Musclow.

Reagents and preparation of the solution:

Sodium citrate	6.25g
Sodium chloride	2.50g
Cetrimide powder	16.70g
Potassium cyanide	125.0 mg
Potassium ferricyanide	41.7 mg
Potassium dihydrogen phosphate	166.7 mg
Distilled water to	1000.0 ml
Brij-35 or Nonidet P40	1.0 ml
Formaldehyde 10% (Formalin 25%)	0.5 ml

Mix well, preferably for one or two hours with a magnetic stirrer, and filter once through a 1.2μm Millipore filter and once through a

0.5μm Millipore filter. Dispense into brown glass bottles, label as containing cyanide and keep at room temperature in a dark cupboard. The pH of the solution should be 8.0.

The reagent has now been in use in our laboratory for four months and has proved entirely satisfactory. However, more regular cleaning of the apertures has been found necessary. The chemicals used for 1 litre cost about 50c and considerable economy can be achieved in a busy laboratory.

K. R. Metcalf.
Pathology Department,
National Women's Hospital.

April, 1974.

REFERENCE

1. Skinnider, L. F., and Musclow, E. (1972). *Amer. J. clin. Path.* **57**, 537.

Precaution in Estimating α_1 foetoprot

α_1 foetoprotein is the predominating protein in the early stages of the foetus and its level exceeds those of albumen and transferrin. It disappears completely in the first week of neonatal life but may reappear in the serum of infants up to one year of age who have non-neoplastic disease such as hepatitis. It is used as a screening test for primary hepatocellular carcinoma and is reported as being positive in 60-70 percent of patients with histological or clinical confirmation of the disease.

Various methods have been used to measure this protein including immunodiffusion, Laurell's electroimmunodiffusion and radioimmunodiffusion. The last one is particularly sensitive and can detect the μg levels present in normal adults. Radial immunodiffusion is very simple and consists of placing 5 μl of serum in a well, cut in agar containing specific antiserum. Quantitation is carried out by processing dilutions of a standard sera containing α_1 foetoprotein and comparing the diameters of the diffusion rings.

We have used this method over the past two years where the diagnosis included the possibility of primary hepatocellular carcinoma. This disease has a very low frequency and only one positive has been found in this period and in fact was nearly missed. At first glance there did not appear to be any precipitate but on closer examination there seemed to be a very faint diffuse ring. On the assumption that excess antigen was having a solubilising effect, dilutions were prepared and the test repeated. This gave a positive result with a concentration of about 80 mg/dl.

The serum was from a ten-year-old boy with an enlarged liver and history of a hepatitis-like illness two or three years previously. For various reasons the diagnosis of neuroblastoma had been favoured but biopsy of the liver revealed hepatocarcinoma. In view of this experience it would seem prudent to employ a dilution of the serum as well as the neat serum when using this technique.

R. D. Allan,
Diagnostic Laboratories,
Dunedin Hospital.

From a paper read to the South Island Seminar, Timaru, May 1974.

REFERENCES

1. Hirsch-Marie, H. and Huguet, C. (1972). *Digestion,* **7,** 156.
2. Hoechst, M—Partigen immunodiffusion plates for α_1 foetoprotein.
3. Skovronsky, J. (1971). *Postgrad. Med.,* **49,** 63.

Correspondence

Sir,

We have recently adopted a kinetic creatinine assay utilising the Jaffe reaction described by Lustgarten and Wenk, 1972 (*Clin. Chem.* **18,** 1419).

During the investigation of the method prior to its introduction we noted that timings had to be absolutely consistent for all samples. We found that a standard of 5 mg/dl was most suited for routine procedure. The accuracy and precision of the method were better than the classical manual method with an added advantage in only requiring 0.1 ml of sample per test.

We have been very satisfied with the performance and deem it worthy of consideration by small manual laboratories.

G. F. Beattie,
Laboratory,
Nelson Hospital.

January 1974.

Abstracts

Contributors: D. G. Bolitho, Lexie Friend, Lynette Gazeley. J. Hannen, B. McDonald, and A. G. Wilson

CHEMICAL PATHOLOGY

Clinical Aspects of the Need for High Capacity Analysis. Watts, R. W. E. (1973). *Ann. clin. Biochem.* **10,** 95.

Acceptable standards of medical and surgical diagnosis and treatment depend upon the ready availability of high capacity chemical analysis. This need is expanding and there are valid reasons for believing that it will continue to do so. The present and future needs arise from clinical problems. The efflorescence of clinical chemistry during the past two decades has resulted from these problems and not from autonomous growth which then stimulated further demands from the bedside. The application of chemistry to medicine presents special problems in the fields of (1) data interpretation, (2) data handling, (3) the need to incorporate a wide ranging consultative function into the system. Adaptability as well as new instruments will be needed in laboratory and clinical practice if we are to meet our patient's needs as clinicians and as laboratory workers.

Author's summary.

Evaluation of the IL 138 Clinicard Analyser. Taylor, J. and Joyce Bell (1973). *Ann. clin. Biochem.* **10,** 111.

An assessment of the performance of the IL 368 Clinicard Analyser is given. The methods tested were urea, sugar, bilirubin, aspartate transaminase, and creatine kinase. Overall a good precision and correlation with the routine laboratory methods was found. The machine should prove useful for the analysis of frequently requested emergency determinations.

Author's summary.

Serum Osmolality and its Applicability to Drug Overdoses. Glasser, L., Sternglanz, P. D., Combie, Joan, and Robinson, A. (1973). *Amer. J. clin. Pathol.* **60,** 695.

The use of serum osmolality and serum delta osmolality in drug overdose has been studied. Osmolality determinations were performed on patients with overdoses of ethanol, methanol, isopropanol and a wide variety of drugs including amitryptyline, barbiturates, chlorpromazine, diazepam, morphine, salicylates, and turpentine.

It was found that intoxicants which have low molecular weights and reach high serum levels such as ethanol, cause a marked increase in serum osmolality and serum delta osmolality. However, most drugs have high molecular weights and do not reach high serum levels and thus do not affect the osmolality.

L.R.F.

Gamma-glutamyl Transpeptidase in Diseases of the Liver and Bone. Betro, M. G., Oon, R. C. S., and Edwards, J. B. (1973). *Amer. J. clin. Pathol.* **60,** 672.

A study has been done on the usefulness of serum gamma-glutamyl transpeptidase in patients with a raised alkaline phosphatase (AP). It was found to be a useful test for the differentiation of a raised AP of bone origin from that of hepatic origin.

L.R.F.

Control Materials for Clinical Biochemistry. Technical Bulletin No. 29. Stevens, J. F. (1973). *Ann. clin. Biochem.* **10,** 133

This updates the previous bulletin on this subject (1970). Summary data on all assayed, unassayed, urine and CSF control material available in the UK are given. Details of protein standards are also given.

A.G.W.

Notes on the Quality of Performance of Serum Cholesterol Assays. Technical Bulletin No. 30. Brown, S. D. (1973). *Ann. clin. Biochem.* **10,** 146.

A survey of analytical methods is given and performance obtained in various Quality Control surveys. Because of the lack of accuracy and precision of existing automated methods the author concludes that until a radically new method is evolved, such as an enzymatic method, a simple established procedure such as Zak be used without empirical correction for interference by bilirubin.

A.G.W.

Evaluation of an Improved Pneumatic-Tube System Suitable for Transportation of Blood Specimens. Pragay, D. A., Edwards, L., Toppin, M., Palmer, R. R., and Chilcote, M. E. (1974). *Clin. Chem.* **20,** 57.

This describes the use of a pneumatic-tube system in use in a new hospital in the USA to transport blood samples to the laboratory. A number of constituents were tested to determine if the system caused alteration to them. No significant changes were found. This was attributed to an efficient deceleration device.

A.G.W.

Design and Operation of a Signal Comparator to Increase Efficiency of Continuous Flow Analysers. Neely, W. E., Wardlow, S., and Swinnen, M. E. T. (1974). *Clin. Chem.* **20,** 78.

The design and operation of a signal comparator is described. The bubbled stream is allowed to pass through the flow cell and the signal comparator eliminates the bubble artifact. Because the bubbles pass through the flow cell improved wash is obtained allowing faster sampling rates. Components cost less than US $200.

A.G.W.

Detection, Characterisation and Diagnostic Significance of Human Pregnancy-Associated Glycoproteins. Bohn, H. (1973). *Blut.* **26,** 205.

Two pregnancy-associated glycoproteins (SP_2 and SP_3) are classified as " reactants of the acute phase ", They occur in trace amounts in all or almost all sera from normal subjects; elevated levels have been found during pregnancy as well as in patients suffering from a variety of diseases. It is proposed to designate the pregnancy-associated glycoproteins as AP(acute phase)-glycoproteins. Their detection might prove to be valuable in differential diagnosis as well as in assessing the activity of a disease process and the effectiveness of therapy. The antisera used for their detection by Ouchterlony gel

diffusion was prepared by immunising rabbits with protein fractions from human placentas. The pregnancy-associated glycoproteins resemble C-reactive protein, but they are not identical.

SP_2 and SP_3 tests were positive in about 80 percent and 70 percent respectively of the sera from patients with malignant tumours as well as from patients with non-neoplastic diseases. In most cases of leukaemia, however, the proteins showed no or only slight increases. At present there is insufficient data to ascertain whether the detection of pregnancy-associated glycoproteins will be applicable and useful in differential diagnosis.

J.H.

Turbidimetric Estimation of Chylomicrons and Very Low Density Lipoproteins in Human Sera after Precipitation by Sodium Lauryl Sulphate. Burstein, M, and Scholnick, H. R. (1973). *Biomed. Exp.* **19** (1), 16.

With the appropriate concentration of sodium lauryl sulphate (SLS), specific precipitation of trigylceride-rich lipoproteins occurs in human serum at 35°C.

The reagents were 3 percent NaCl and 10 percent SLS. A Perkin-Elmer linear absorbance spectrophotometer (Coleman, Model 44) was used. Fresh serum, 0.5 ml, was added to 1.5 ml of the NaCl solution in a calibrated cuvette; the optical density was read at 700 nm (serum blank) and 0.05 ml of the SLS solution was added. After incubation for 30 min in a water bath at 35°C, the optical density was read again.

In normal fasting serum the blank is low (OD × 100 = 0.5-1.0) but in lipaemic serum it may be very high (20 or more); for this reason, in the case of lipaemic sera, the blanks are not subtracted.

J.H.

Alkaline Phosphatase in Pleural Effusion. Doust, J. Y., and Kohout, E. (1973). *Israel J. med. Sci.* **9**, 1588.

Results of current methods for pleural effusion investigation, such as determinations of protein, glucose, specific gravity and cytologic examination, are not conclusive in all cases. The authors report the value of determining alkaline phosphatase activity (Bodansky's method was used, normal 1.5-5.0 units) in establishing the aetiology of plural effusion. Elevated values (>10 units) were found in pleuropulmonary malignancy, pulmonary infarction and amoebiasis. In tuberculosis, alkaline phosphatase activity was absent in 10 cases and amounted to <2 units in 12 cases.

Elevation of alkaline phosphatase in pulmonary infarction seems to be significant and conclusive. The authors strongly recommend determination of this enzyme since this is a valuable contribution to the recognition of a condition for which clinical diagnosis is notoriously difficult.

J.H.

HAEMATOLOGY & IMMUNOHAEMATOLOGY

The Complement System. Mayer, M. M. (1973). *Scientific American,* November, 54.

The author graphically describes the action of complement and the resultant perforation of the cell membrane. Artificial structures called Lipsomes which mimic the bilipid structure of the cell membrane are used in the electron microscopy studies of the lesions made by the complement. There is also electron micrographs of the subunit $C1_q$ showing its actual shape. The Properdin Pathway for activation is also discussed in some detail. The other activities of complement are also discussed.

B.M.

A Papain-Bromelin-Polybrene Four Channel Autoanalyser System for Blood Group Antibody Screening. Habibi, B., Gerbal, A., and Salmon, C. (1973). *Vox Sang.* 25, 289.

The authors developed a 4-channel autoanalyser system for antibody screening using low ionic strength low pH polybrene method at 22 and 18°C; Bromelin methylcellulose method at 22°C; and Papain methylcellulose method at 18°C. This system was designed to be used by a transfusion service for its routine screening of serum, and to this end an accelerated sampling rate was used. During 12 months 22,912 sera were screened by this system and also by manual techniques. The autoanalyser system's performance with respect to natural antibodies was not outstanding, missing 26 percent Lewis and 23 percent P_1 antibodies on all four channels. However, immune antibodies were detected more easily than by manual techniques. The results indicate that using the autoanalyser system in conjunction with simplified manual screening appears to be practical.

B.M.

Assessment of Coagulation and Fibrinolysis in Pre-eclampsia. Wood, S. M., Burnett, D., Picken, A. M., Farrell, G. W., and Wolf, P. (1974). *B.M.J.* 2, 145-149.

A method is described for distinguishing coagulation from fibrinolysis by three estimates of fibrinogen. This " fibrinogen series " together with plasma antithrombin and urinary urokinase have been compared in pregnant patients with venous thrombosis and pre-eclampsia. Evidence is presented for active coagulation during deterioration of the pre-eclampsia state and for enhanced fibrinolysis during improvement.

L.G.

MICROBIOLOGY

Granulocytes Containing Cytoplasmic Inclusions in Human Tuberculous Pleuritis. Faurschou, P., and Faarup, P. (1973). *Scand. J. resp. Dis.* 54, 341.

Granulocytes containing cytoplasmic inclusions were consistently found in the pleural fluid from 10 patients with tuberculous pleuritis. The pleural fluid was centrifuged at 2100 *g* for 10 min. Fractions of the sediment were stained supravitally as described by Jackson (1954). The inclusions were 1-4 μm in diameter, were highly refractile and could easily be identified even in unstained specimens. Isolated inclusions were frequently found in the pleural fluid. In addition, in fixed specimens " unclear dust " from disintegrated granulocytes could sometimes be seen in conventional slides from the sediment.

J.H.

The Use of the Unstimulated Nitroblue Tetrozolium Test as a Routine Screening Test for Bacterial Infection in an Adult Population. Bittner, S. J., Kieff, E., Windhorst, D., and Meier, P. (1973). *Am. J. clin. Pathol.* **60**, 843.

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tetrazolium test potentially adaptable for routine general hospital use was devised and employed to study 141 subjects in four population groups with four different health states. It was found that the unstimulated NBT test may be only somewhat useful as an adjunct to other indices in diagnosing bacterial infections.

L.G.

Anaerobic Infections in Chronic Prostatitis and Chronic Urethritis. Justesen, T., Nielsen, M. L., and Hattel, T. (1973). *Med. Microbiol. Immun.* **158,** 237.

Forty-six patients with chronic prostatitis or chronic urethritis were examined for anaerobic infections of the urine and prostate. The technique of anaerobic culture was based on a transport time for the clinical samples of $<\frac{1}{2}$ h, as well as the use of the "glove box" technique and pre-reduced media. Anaerobic bacteriuria was found in one of the 46 patients, but in no case were anaerobic bacteria localised with certainty to the prostatic tissue, the prostatic secretion or the urethra.

Although the study does not involve extensive material, the results do not support the claim that anaerobes play a common role in patients with chronic prostatitis, without demonstrable anaerobic bacteria in the prostatic secretion. Similarly, the results do not provide any support for the view that in patients with chronic urethritis, the prostate is invaded by aerobic or anaerobic bacteria.

J.H.

Bacteriology and in vitro Antimicrobial Susceptibility of the *Pseudomonas fluorescens* group Isolated from Clinical Specimens. Morton, W. J., Maken, M. D., and Washington, J. A. (1973), *Amer, J. clin. Path.* **60,** 831.

The biochemical characteristics, sites of inoculation and antibiotic susceptibility of 183 isolates of *Pseudomonas fluorescens* and *Pseudomonas putida* from human sources are described. The most important site of isolation was the respiratory tract. No differences in antibiotic sensitivity were noted between the two organisms both of which were resistant to Carbenicillin and sensitive to Gentamycin, Kanamycin, and Polymixin B.

D.G.B.

Evaluation of a Bacteriuria Screening Test in a Large Hospital Environment. Senterfit, L. B., and Baldridge, P. B. (1973), *Amer. J. med. Technol.* **39,** 454.

A trial of a modification of the Dip-Slide technique for semi-quantitative urine counts in comparison with a calibrated loop technique is described. The Dip-Slide in this case consists of a commercially prepared disposable "paddle" containing Trypticase Soy and E.M.B. agars side by side and contained in a disposable vial. The paddle is "dipped" and counted in the usual way. As with earlier trials of dip inoculum techniques the results compared favourably with the calibrated loop method. No particular advantage appears obvious comparing this proprietary product with simple agar counted slides.

D.G.B.

Testing a Steam-formaldehyde Steriliser for Gas Penetration Efficiency. Line, S.J., and Pickerill, J. K. (1973), *J. clin. Path.* **26,** 716.

An ingenious and apparently effective device for the testing of low temperature sterilisers is described. Full constructional details are given.

D.G.B.

Incidence and Isolation of Bacteroides Species from Clinical Material and their Sensitivity to Antibiotics. Mitchell, A. A. B. (1973), *J. clin. Path.* **26,** 738.

The results of a one-year survey to determine the frequency of Bacteroides infections is presented. The authors conclude that it is well worthwhile to make a special search for these organisms in vaginal swabs, pus swabs from deep seated wounds and swabs from infected ears. The isolation rate from vaginal swabs is particularly impressive (17.6 percent), although the difficulty of deciding whether these organisms are pathogens or commensals is stressed. The paper contains a useful observation on the time taken for metal anaerobic jars to reach 37°C and makes suggestions to overcome this.

D.G.B.

An Outbreak of Infantile Gastroenteritis due to *E. coli 0142.* Kennedy, D. H., Walker, G. H., Eallon, R. J., Boyde, J. F., Gross, R. J., and Rowe, B. (1973), *J. clin. Path.* **26** 731.

The investigation of an outbreak of infantile gastroenteritis is described. There were 12 cases with two deaths. The investigations are fully described and the paper is essential reading to all concerned with cross-infection control in hospitals.

D.G.B.

Characterisation of Pseudomonas Species for Identification in the Clinical Laboratory. Johns, P. A., and Tschen, R. G. (1973), *Amer. J. med. Technol.* **39,** 12, 495.

An identification scheme using 13 diagnostic tests is presented. An evaulation of the value of many of the competing techniques for determining these 13 characteristics is also given.

D.G.B.

Multiple Loop Inoculator as an Aid in Bacteriocine Typing Techniques. Brown, D. O. (1973), *Med. lab. Technol.* **30,** 4, 351.

A simple and cheaply constructed multiple inoculator is described.

D.G.B.

The Role of Extracellular Slime Secretion in the Swarming of Proteus. Fuscoe, F. J. (1973). *Med. lab. Technol.* **30,** 373.

An investigation into the swarming phenomena. The author concludes that these organisms secrete an extracellular slime which acts as a lubricant to facilitate swarming.

D.G.B.

The Influence of Carbon Dioxide on the Growth of Obligate and Facultative Anaerobes on Solid Media. Watt, B. (1973), *J. med. Microbiol.* **6,** 307.

An investigation is described which was designed to determine whether the often advocated addition of CO_2 to H_2 in anaerobic systems is in fact of practical benefit. The author, besides testing the enhancement of growth of clostridial and bacteroides species investigated two strains of *Streptococcus pyogenes* both for enhancement of growth and of haemolysis. The author concludes that the addition of CO_2 to hydrogen anaerobic systems is in fact beneficial and in particular enhances the haemolytic properties of the *Streptococcus pyogenes.*

D.G.B.

Agar Plate dilution method for routine antibiotic susceptibility testing in a Hospital Laboratory. Haltalin, K. C., Markley, A. H., and Woodman, E. (1973), *Amer. J. clin. Path.* **60**, 384.

An agar plate dilution method which gives antibiotic susceptibility results as MIC's and is as suitable for routine use as disk diffusion techniques is described. The method appears to be fairly cheap and requires little specialised apparatus except a replicating apparatus. The authors admit to some difficulty in standardisation and if these could be overcome this method appears to have a certain attraction as a method of antibiotic susceptibility testing.

D.G.B.

Systemic infection due to ***Actinobacillus actinomycetemcomitons.*** Burgher, L. W., Loomis, W., and Weir, F. (1973), *Amer. J. clin. Path.* **60**, 412.

The description of a case having abscesses due to *Actinobacillus actinomycetemcomitans* situated in the thyroid, the parietal region and in the soft tissue overlying the left ribs. The patient was severely ill though he recovered after appropriate antibiotic treatment. This is only the third human case of infection with this organism reported. A summary of the growth requirements and biochemical characteristics of the organism are given.

D.G.B.

A new Fluorescence and Kinyouns acid fast stain. Lavallee, P. W. (1973), *Med. J. clin. Path.* **60** 432.

A combination stain using auramine O and cold carbolfuchsin staining technique for alcohol fast bacilli is presented. The paper describes a new commercially prepared combination of these stains. Although the author compares this new technique with the method advocated by Fussillo and Burns in the same Journal in 1968 he inexplicably fails to quote this in his list of references. The new method is certainly quicker than that described by Fussillo and Burns, and Lavallee considers that, in addition, the results are superior to the older method.

D.G.B.

Pathogenic Lactobacilli. Sharp, M. E., Hill, L. R., and Lapage, S. P. (1973), *J. med. Microbiol.* **6**, 281.

This paper describes the investigation of seven strains of lactobacilli which appear to be of pathological significance. There were four strains of *Lactobacillus casei* var *rhamnosus,* two stains of *L. plantarum,* and one strain of lactobacillus species subgenus thermobacterium. Full details of the identifying characteristics of these organisms is given and the authors point out that the isolation of lactobacilli from clinical material, particularly blood cultures may well be of clinical significance and that these organisms cannot be dismissed as contaminants.

D.G.B.

Endocarditis Caused by ***Bacillus subtilis.*** Reller, J. B. (1973), *Amer. J. clin. Path.* **60**, 714.

A proven case of endocarditis due to *Bacillus subtilis* is described. The patient was a drug user. This isolate demonstrates the importance of not automatically dismissing Bacillus species as non pathogenic when isolated from multiple blood cultures.

D.G.B.

Dye Impregnated Paper Strips for Staining Bacteria. Elston, H. R., and Quigley, J. (1973), *Amer. med. lab. Technol.* **60**, 476.

The method of use of proprietary dye impregnated strips is described and an evaluation of their effectiveness as compared to traditional methods made. The method appears to be more time consuming and less easy than traditional techniques, it has nothing to commend it.

D.G.B.

Flavobacterium meningosepticum **from Cases of Meningitis in Botswana and England.** Lapage, S. P., and Owen, R. J. (1973), *J. clin. Path.* **26**, 747.

This paper describes the clinical course of two new cases of infection by this rare pathogen. The organisms resistance to most, commonly used antibiotics is stressed.

D.G.B.

Miscellaneous Gram Negative Bacilli: Key to their Identification. Porres, J. M. (1973), *Amer. J. med. Technol.* **39**, 402.

This paper presents a new key for the identification of aerobic gram negative rods (excluding members of the Enterobacteriaceae). The author points out the difficulty of keeping such keys up to date and stresses the need to keep abreast of nomenclatural changes. The key appears to be an advance on those prepared by Cowan and Steele and uses simple media and identification tests.

D.G.B.

Human Meningoencephalitis caused by Free Living Amoeba. Cotter, D. A. (1973), *Amer. J. med. Technol.* **39**, 417.

A brief review of this condition is presented together with a description of the causative organism *Naegleria fowlerii.* The author stresses the important point that *Naegleria fowlerii* can be selectively isolated from water and mud by incubation of cultures at 43°C, this temperature being lethal to non-pathogenic amoeboid species.

D.G.B.

Book Reviews

Auto-immunity and Auto-immune Diseases.

Sir MacFarlane Burnet (1972). 243 pages with some illustrations. Medical and Technical Publishing Co. Ltd. Price, $NZ7.30, N. M. Peryer Ltd., Christchurch.

Sir MacFarlane Burnet is the originator of the clonal selection theory which has provided the main stimulus for immunological research in the last fifteen years.

The tone of the book is reflective and interpretative rather than specific. It is not a textbook providing a systematic exposition of the subject nor in the words of the preface, " intended for the professional immunopathologist but rather for those with a peripheral interest wanting a general perspective on the subject and its interpretations."

Autoimmunity is here considered as a process of Darwinian selection amongst the circulating lymphoid cells of the body. Thus there is random production of cell types which may be eliminated in an unfavourable environment. Specifically, immunocytes must be eliminated which react with accessible body antigens. Should an immunocyte undergo mutation it may be resistant to elimination and proliferate to form a clone, that is a homogeneous collection of identical cells. This constitutes a " forbidden clone " which is the basis of auto-immune disease. The introductory chapters discuss the relevant biological background to this theory. Somatic mutation can be physiological and this is exemplified by local pigmentation or freckling whereby a clone of pigmented cells selectively develops in response to an environmental hazard, i.e., the social obligation to acquire a tan! Moles which are congenital accumulations of melanocytes may also undergo a somatic mutation under the influence of sunlight and give rise to a malignant melanoma. It has been suggested that such mutations may be the way in which malignant tumours originate. The probability of this occurring can be assessed because if large numbers of cases are considered, the frequency can be shown to conform to a pattern. (Stochastic approach.)

The idea of natural selection carries with it the concept of an infinite variety of antibodies or cells capable of producing antibodies when stimulated, rather than the classical concept of a specific antibody for a specific antigen. The situation is much less precise and simple and there are degrees of affinity.

The origin, function, and interaction of T-cells and B-cells are discussed at some length, in regard to modern theory, tolerance, and pharmaceutical aspects of immune responses.

A chapter of the book is devoted entirely to the New Zealand mice strains as models for investigation of auto-immune disease. Many familiar names are mentioned in this context; Sir Charles Hercus, Dr Marianne Bielschowsky, and Mr W. H. Hall. Three initial strains were developed from mice of a mixed colour stock brought to New Zealand in 1930 from the Imperial Cancer Research Fund laboratories in London. These three strains, black, chocolate, and white or NZB, NZC, and NZW or their hybrids suffer from a genetic predisposition to develop auto-immune haemolytic anaemia and other manifestations of auto-immune disease. Apart from the general conclusion that the anomaly is an abnormally high resistance of all immunocytes to destruction or inhibition by specific antigenic contact the high hopes that investigation of these laboratory models would solve the nature and pathogenesis of the disease have not yet been realised. There is considerable discussion of a speculative nature on these animals. There are chapters on auto-immune haemolytic anaemia, generalised and localised auto-immune diseases and the pathogenesis of auto-immune disease.

Finally potentialities and limitations of theory, the significance of auto-immunity for ageing and a programme for the future are discussed.

While this book is not intended for the specialist it is certainly not for the uninitiated.

For those interested in the subject, it would probably prove rewarding.

R.D.A.

The Application of Comparative Morphology in the Identification of Intestinal Parasites.

John W. Moose, M.S., L.T.C., A.U.S. (Ret.) 1973. Published by Charles C. Thomas, Springfield, Illinois. (The American Lecture Series in Clinical Microbiology.) Price, $US7.50.

This text is composed of 40 pages of colour photomicrographs (136) of all the relevant protozoan and helminth parasites and is obviously designed as a bench manual. Although it is designed as a diagnostic aid it is intended to be complemented by other suitable parasitology textbooks.

Each photograph carries underneath the name of the parasite, the stage of development, magnification, the stain employed, and relevant data which assist in identification. The first half of the manual is devoted to protozoans, which include the more consistently found stages of development of Entamoeba, Endolimax, Iodamoeba, Chilomastix, and Giardia. Interspersed amongst these photomicrographs are some of the more commonly confused pseudoparasites such as neutrophils and blastocystes species. Some of the stained examples of trophozoites and cysts are slightly obscure and do not relate to the descriptions given, for example, the chromatoidal bodies in one picture are very hard to pick out. It is perhaps unfortunate that these fine photomicrographs could not have been reproduced on a larger scale. The iodine preparations demonstrated are extremely useful to the laboratory technician and the teacher.

The second portion of this text deals with the range of intestinal helminths most likely to be found not only endemic, but carried by visitors to this country. I think a more obvious division could have been made in the text between protozoans and helminths. Two complete pages are devoted to *Ascaris lumbricoides* and some particularly fine examples of the various stages, both fertilised and unfertilised, corticated and uncorticated, are shown. After seeing these, no one should miss an ascaris in a faeces sample. *Trichuris trichiura* also is given similar treatment. Hookworm eggs are not the best examples and could have been better represented. Pseudoparasites, plant cells, fibres, and pollen grains demonstrate clearly some of the problems the parasitologist must contend with. The examples of rhabditiform larva of hookworm and strongyloides demonstrate very well the variation in buccal groove which is a diagnostic aid.

Although the only way to gain expertise in identifying worm parasites is to spend a great deal of time studying samples, this text fills a position in the laboratory technologist's library as a diagnostic aid.

I feel that perhaps the title could have been somewhat simplified to " An Aid to Identifying Intestinal Parasites " yet still have retained its meaning

B.M.C.

Bacteraemia, Laboratory and Clinical Aspects.

A. C. Sonnenworth, Ph.D., 1973. Charles C. Thomas, Publisher, Springfield, Illinois. 106 pages. Price, $US7.95.

The idea for this book stemmed from a seminar on bacteraemia held at the 1971 annual meeting of the American Society for Microbiology. Six chapters, each dealing with a different facet of bacteraemia, are authored by experts in their particular field.

Dr A. Sonnenworth in chapter one outlined the extent of the problem. In 1968 it was estimated that there were 10 cases of bacteraemia per 1,000 admissions. At this rate there were at least one-quarter million cases in the United States with a minimum of 50,000 deaths.

A survey of blood culture techniques and interpretation of results in twenty-one proficient clinical laboratories is discussed by Dr Bartlett. Although a wide variety of methods was found, the majority of laboratories were using acceptable techniques with the exception of two, one which omitted an anaerobic method and the other which did not subculture at all.

Dr S. Finegold stresses the importance of early detection of bacteraemia and states that optimum results can only be obtained when both clinical and technical problems are realised. The combination of a membrane filter system and an anaerobic osmotically stabilised broth is described as an ideal system and its advantages are discussed.

Chapter four by Dr J. Washington deals with the ever-increasing problem of anaerobic, unusual and fastidious organisms responsible for bacteraemia at the Mayo Clinic. A staggering 20 percent of bacteraemias were caused by anaerobes, mainly bacteroides, in a two and a half year survey.

Richard Rosner's chapter deals with the

relationship between the physical condition of bacteria when present in the patient's bloodstream and the ability of the laboratory to recover such organisms. It also attempts to explore the possible interactions between the organisms, the patient's blood and the blood culture system. An experiment comparing Brucella broth with and without liquoid and liquoid plus sucrose is described. Results show that the osmotically stabilised broth with the addition of liquoid greatly enhances the recovery of organisms from the blood. It is interesting to note that most of the laboratories in the survey did not use a hypertonic medium routinely. I am sure the same trend would be found in a New Zealand survey.

The final chapter by Drs Maki, Rhame, Goldmann, and Mandell discusses the infection hazard posed by contaminated intravenous infusion fluids.

To the reader's advantage is the long list of up-to-date references at the end of each chapter.

Although much of the material presented is not new, results indicate that a new look at existing methods is worthwhile. No matter how efficient one's method of culturing blood may be I am sure there is always room for improvement. At the very least this book provides food for thought.

M.J.

SPUTUM—Fundamentals and Clinical Pathology. Compiled and Edited by Mauricio J. Dulfano, M.D. 632 pages, illustrated. Charles C. Thomas, Publisher, Springfield, Illinois, U.S.A.

Fifteen of the 18 contributors to this book are American physicians and scientists, with chapters by two Belgium physicians and a Canadian pharmacologist.

The idea of a text about sputum is commendable. However, this book has several disappointing features. In the copy submitted for review 10 of the 15 chapters have their page numbers given incorrectly in the list of contents, and the index also has many items listed with wrong page numbers. This printing error is obviously irritating for readers.

The microbiology examination is one of the commonest and most important clinical pathology tests performed on sputum but in the book only 16 pages are devoted to bacteriology and 40 pages for mycology. Thus it is not surprising to find the recently devised techniques for quantitative estimation or organisms in sputum completely omitted.

Although the physical properties of sputum and the mechanisms of respiratory tract clearance are important aspects of sputum the 42 pages and 73 pages given to these subjects respectively, must be regarded as disproportionate.

Of the three colour plates in the book, the one showing Gram stains of sputum is an extremely disappointing reproduction. The tubercule bacilli in the acid fast stain are virtually invisible rather than as quoted "faintly stained". The plate of Papanicolaou stains of cells in sputum is of a better standard.

Words such as "chocolatized, maximize, fluidify, aerosolisation, and sputology" used in the text are unnecessary tongue twisters and surely "methodological sophistication" would be improved by the 28 percent reduction in letters to read, sophisticated methods.

There are useful chapters on the anatomy of the mucocilliary apparatus, ultrastructure of the respiratory mucosa, electron microscopy of sputum and the action of pharmacological agents on respiratory fluid. The authors of these have added their own experimental findings where appropriate, and the chapters are illustrated with good quality black and white photographs and relevant tables and graphs.

The information given in the chapters dealing with non-malignant exfoliative cytology and cancer cytology is probably adequate for most respiratory physicians and laboratory technologists but the details are inadequate for histopathologists.

There is an interesting account of sputum proteins with an extensive review of the literature. The description includes work on lactoferrin, immunoglobulins, lysozyme, kallikrein, and mucin in sputum. Unfortunately much of this information has no practical application to chemical pathology.

The chapter entitled special examinations of sputum is of practical value for laboratory workers. Techniques for demonstrating iron and asbestos bodies, lipid, cholesterol and mineral oil, aspirated foreign material, parasites and larvae in sputum are given.

The references in many American textbooks are totally from American publications and so it is pleasing to see that the authors of "Sputum" have quoted from many international sources.

This book contains material useful to many workers who will have occasion to study and

examine sputum. Personnel in specialised work within pathology, microbiology, physiology, and pharmacology will often need the more detailed texts available in their respective disciplines.

R.S.

Computerizing a Clinical Laboratory. 1973. Jerry K. Aikawa, MD, and Edward R. Pinfield. Published by Charles C. Thomas, Springfield, Illinois, USA. 96 pages with some illustrations. Price $US8.75.

" The story of how the University of Colorado Clinical Laboratory Computer System came to be," would be a suitable subtitle for this book; I nearly wrote " novel ", and indeed there are elements of suspense, drama and human interaction in its pages.

The laboratory started with a single channel of an autoanalyser in 1957 and by 1965, 85 percent of the work was fully automated. At this time there was a great increase in concern over quality control and data processing and this was a universal occurrence. An IBM 402 unit record accounting machine was obtained to assist in organising the data. It is interesting to read of the total involvement of the staff in the further development of the system. All the staff took a written aptitude test in data processing and were assigned jobs on this basis. Instruction then followed in computer concepts and Fortran computer language and it became apparent that only a few people had the innate ability and talent to converse fluently with the computer. Systems analysis (a formal system of problem solving) was used to see how the functions of the clinical laboratory could best be served by data processing.

The next step was to obtain an IBM 1800 process control computer. This was eventually obtained after overcoming a hazard referred to in computer marketing circles as " slippage ". This term indicates an inability to meet scheduled delivery dates—not an uncommon phenomenon! In spite of 16K core memory, the peak-picking programme was too large and a slope detector and interrupt signal device had to be designed locally. Following the mode used with the early IBM machine the system was first used " off-line ", but this proved inefficient. Subsequently an " on-line " real-time system was devised and the core capacity increased to 48K.

The possibility of computerising microbiology and haematology data has also been explored with some success. Original microbiology data in code form (implying that a standard scheme for identifying and describing micro-organisms had been agreed upon) were keypunched. This took too long and this was replaced by a punch activated by an optical scanner. At the time of writing it appeared that a scheme for in-putting haematology data had been prepared using the Coulter S and a machine designed to in-put differentials and cell descriptions, but that hardware was awaited.

In the discussion it is interesting to note the authors' opinion on laboratory staffing. They say, " there is little need for a physician in the management of the day-to-day functions of a clinical laboratory. The future role of the physician is as a consultant to the clinician . . ."

In conclusion, while this book does not provide a cross-section of available apparatus, and there is at least one publication which does make such a comparison, it does provide a readable account of the manner in which the communication problem arising from automation was successfully dealt with in one laboratory. It should be of interest to those similarly involved.

R.D.A.

Manual for Hepatitis B Antigen Testing. By M. Ashcavai, and Robert L. Peters. Published by W. B. Saunders Company and obtainable from N. M. Peryer, Ltd, Christchurch. 275 pages. Illustrated. Price $NZ5.55.

Since 1968 when a viral by-product was first recognised in the blood of many patients suffering from viral hepatitis a number of techniques have been devised for its identification which has ultimately led to a wide variation in terminology.

The authors' initial intention in this manual has therefore been to consolidate and classify the information that has been accumulated over the past few years and to clarify some of the terminology currently in use.

Although this manual is basically a collection of these various methods currently employed it serves as a welcome addition to the libraries of those people employed in the testing for and identification of hepatitis whether in patients or when screening blood donations for the disease.

The methods listed range from those that may be employed in any small laboratory where facilities exist to those requiring costly

pieces of equipment more suited to research establishments and the choice of the most applicable method in various laboratory situations is discussed.

The authors focus special attention upon the procedural steps in which errors or inaccuracy may be encountered for each method. The authors place special emphasis on these variabilities and stress the need for accuracy and reproducibility which should be of paramount importance for anyone employed in this field.

The methods for identification listed include: Agar Gel Diffusion, Rheophoresis, Counter-electrophoresis, Complement Fixation (both manual and automated), Haemagglutination and Haemagglutination Inhibition, Reverse Passive Haemagglutination and Radioimmunoassay. For each of the aforementioned methods, each devoted a chapter, the materials and equipment are listed together with the procedures, sensitivity, precautions and interpretation of results. A further chapter is devoted to additional methods including electron microscopy, tissue culture and modifications to the previous listed techniques.

The manual is unusual in its final printing in that only one side of each page is used (130 pages of actual print) leaving a blank page presumably for the addition of further notes as the owner requires.

A.E.K.

Parenteral Products. M. J. Groves, M.Pharm., Ph.D., M.P.S., M.Inst.Biol. 316 pages, illustrated. Publisher: Willian Heinemann Medical Books Ltd., London. Price in U.K. £4.00

This book is a late but welcome addition to the shelves of not only producers of parenteral products, but also of those who use them, and one would hope, of those who supply producers with raw materials, equipment, etc. It comes in the wake of fatal incidents due to large volume parenterals, on both sides of the Atlantic which gives it added interest to producers.

This book is a review of published and unpublished material and includes over 350 references conveniently listed at the ends of the chapters in which they are cited. The references are international, but the book relates to the British scene more closely than to any other.

Dr Groves deals with perspectives on the use of parenteral solutions in the opening chapter and then deals with their formulation in a general way. He writes worthwhile chapters on filtration, sterilisation and the working environment and then goes on to an in-depth review on the control, monitoring, size distribution, nature, origin, and hazards of particulate contamination. He concludes with a discussion of pharmaceutical problems associated with the administration of large volume parenterals.

The author has published a lot of work on all aspects of particulate matter in parenteral solutions and this book is valuable if for this aspect alone. One gains insight into the difficulties involved in setting objective standards on the limits of particulate contaminants which many countries are currently endeavouring to do including New Zealand. This book carries informed comment in the field.

Generally the book is concerned more with control than with development or production, more with hospital aspects than with commercial ones, more with protecting the patient than the financial costs, but I hasten to add there is no imbalance. The author wishes the patient to receive a solution clear of contamination from rubber, glass, plastic, personnel, the environment, apparatus and true to label, and his book will assist all concerned to that end. He doesn't provide all the answers but he does indicate directions for further investigations whereby products may be improved.

This will, I believe, be a much-quoted book where solutions are discussed and where solutions are sought. Sadly it may also be quoted as an example of poor proof-reading.

J.J.G.

What's New?

SMALL ELECTRIC FURNACES TO 1100°C IN THREE MINUTES

Ceramic fibre lining provides exceptional thermal insulation and resistance to thermal shock

Small electrically-fired furnaces and kilns, such as those used for firing pottery and enamelling or firing dental porcelains, can now be made many times more efficient by lining them with a ceramic fibre refractory material. One of these materials, known as Triton Kaowool, has such excellent thermal insulating properties that a small dental furnace, for example, may be raised to operating temperature in only three minutes instead of an hour—the time required for dental furnaces with conventional linings.

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The furnace lining comprises an 89mm diameter vacuum-formed tube (or muffle) of ceramic fibre, whose inner surface has grooves that support the heating element. This muffle, which takes less than an hour to fit, keeps the vacuum chamber cool without the need for cooling apparatus.

The low-cost Enamelaire K1 kiln, produced by Enamelaire Ltd, Watford, England, may be used for enamelling, glazing, glass-forming, and metal treatment. It has a firing chamber measuring 152mm × 152mm × 76mm, formed from 10mm thick ceramic fibre board. The 750W heating element is contained within a specially made ceramic tile which serves as the floor of the kiln. The kiln reaches enamelling temperature in 45 minutes.

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For further information, please contact George Gramlich, Beckman Instruments, Inc., 2500 Harbor Boulevard, Fullerton, California 92634, USA.

VOLUNTEER SERVICE ABROAD WANTS PARA-MEDICAL VOLUNTEERS FOR PACIFIC

Two hospitals in the Pacific area need an occupational therapist and a pharmacist to continue essential work involving department administration and local staff training.

Volunteer Service Abroad is looking for qualified people to continue the work of two volunteers returning to New Zealand.

A Rotorua occupational therapist, Helen Hollings, has spent two years working at Moto'otua Hospital, Apia, Western Samoa, where she developed work done in this field and organised a training programme for the local staff. At present there is no one available to lead this work. The hospital is large, with 300 beds, and the volunteer would either share a flat with another volunteer or live in the sisters' quarters at the hospital.

Peter Burdon, Invercargill, returns from Vaiola Hospital, Nuku'alofa, Tonga, in February, leaving a department without a qualified person in charge. The dispensary at this 200-bed hospital is busy and urgently needs someone to take over. The volunteer will share a flat or house with another volunteer.

Qualified and experienced volunteers are also needed at a new British hospital due to open in September in Vila, New Hebrides. A radiographer and a laboratory technologist appointed to the hospital will set up and develop their respective departments in the new hospital and in outlying hospitals on remote islands.

Assignments for doctors, nutritionists, dietitians, and physiotherapists are available in many territories in the Pacific and Asia. Further information and application forms are available from the Selection Officer, Volunteer Service Abroad, P.O. Box 12-246, Wellington.

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

Illustrations

Illustrations are costly and should be used sparingly. Graphs, line drawings and photographs are all referred to as 'Figures' and should be numbered in the order of their appearance in the text using arabic numerals. Drawings (in indian ink on stout white paper) and photographs, should be about twice the size of the actual reproduction. The position of figures in relation to the text should be noted in the typescript. Legends typed on separate sheets are numbered to correspond with the illustrations. Tables should be typed separately and numbered in roman numerals.

Nomenclature and Units

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

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

Length: m, cm, mm, μm, nm.
Area: m^2, cm^2, mm^2, μm^2.
Volume: litre, ml, μl, nl, pl ('litre' in full avoids confusion with 'l')
Mass: kg, g, mg, μg, ng, pg.
Mass concentrations: kg/litre, g/litre, mg/litre, μg/litre. For the present concentrations per 100 ml also accepted as are daily outputs in urine and faeces.
Molar concentrations: mol/litre, mmol/litre, μmol/litre, nmol/litre. (For the present mequiv/litre may also be used.)
Temperature: Express as °C.
Time: s, min, h, d, a. The latter two symbols which stand for day and year respectively are best expressed in full to avoid confusion.
Density: kg/litre (relative density replaces 'specific gravity')
Clearance: litre/s, ml/s (for the present ml/min may also be used).

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