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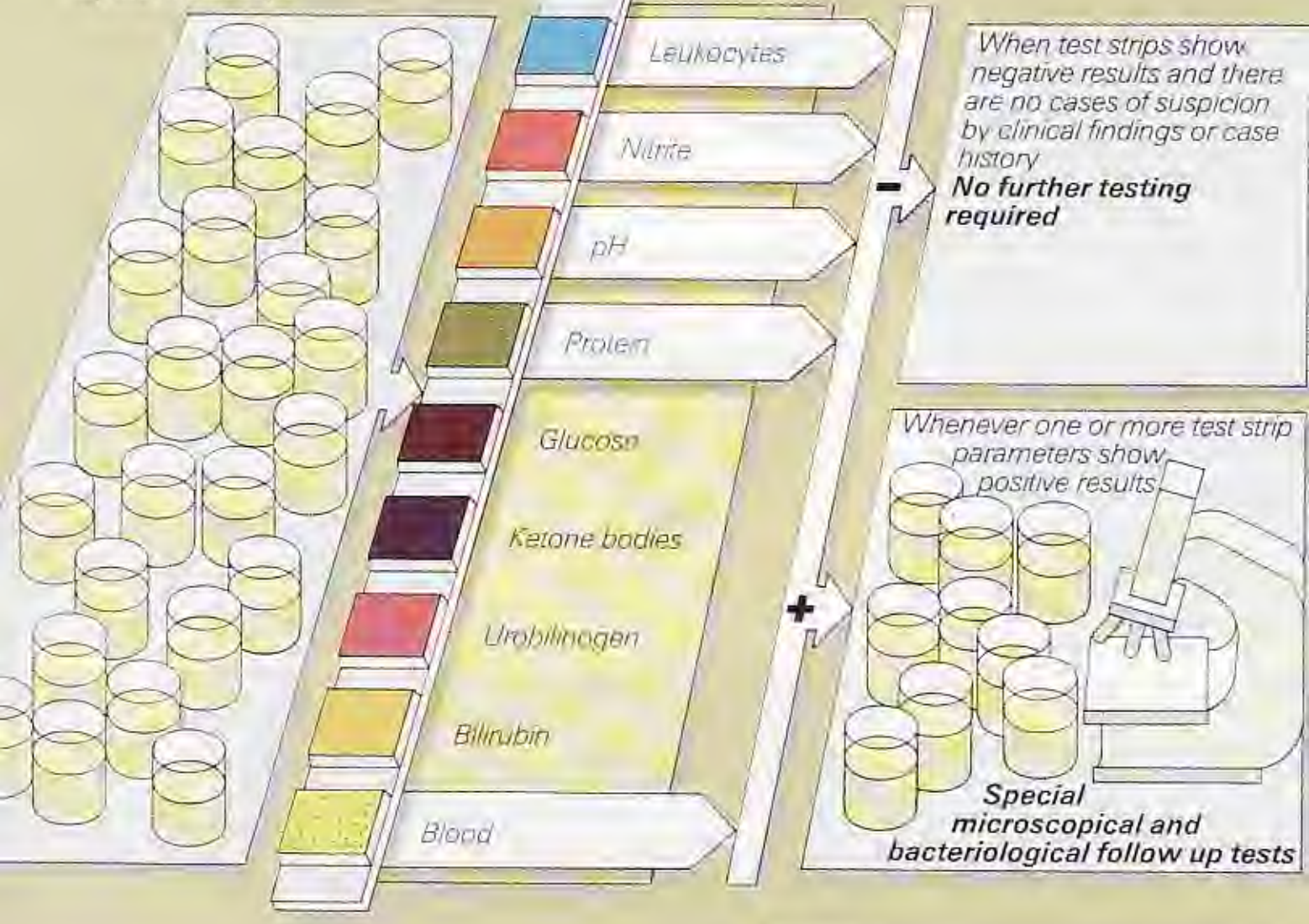

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Dip-Stick Chemistry: Simple and Reliable or Simply Unreliable

W. Hodgson and R.S. McKenzie

Pathology Department, Nelson Hospital, Nelson

The delivery of primary health care to the community is often stated to be the aim of New Zealand's health services and the associated health care industry. Preventive medicine is increasing in importance and there is a heightened community awareness of the health problems associated with life styles and environmental hazards. For those who develop chronic disease there are mutual support groups which encourage understanding of the disease and its management.

Historically the role of a clinical laboratory in health care has been the obtaining, by observation and analysis, of objective information about the health of a patient. Such data may suggest or support a diagnosis and, where appropriate, follow-up tests are used to monitor and assess the efficacy of therapy. More recently, with the development of rapid and reliable techniques for measuring drug concentrations, this latter aspect of our work has increased in importance and we believe that it is appropriate that the contribution of our expertise and technology to the prevention of iatrogenic disease be recognised and vigorously promoted.

Ironically, it is in the long established programmes of diabetes management where this need has become rather urgent. It is obvious that the restrictions placed on the availability of glucose assays by the 8 hr. working day has not been in the best interests of the diabetic out-patient and, since the predictive power of urine testing is known to be less than adequate, it is not surprising that methods for home-monitoring of blood glucose levels have been developed and are now widely used. We believe that our profession must recognise this as but the first of the "do-it-yourself" systems and move rapidly to offer our services as external auditors of these procedures when used at home, at the out-patient clinic and on the ward.

From our experience at Nelson Hospital we will address each of these questions and illustrate our concern in matters of training and quality control. We also propose a sample collection and glucose analysis technique which will allow at least retrospective 24 hour monitoring in an economic and reliable manner.

Monitoring the blood glucose by other than laboratory staff has two aspects, these are self-monitoring by the diabetic patient in their home and work environment and monitoring in the hospital situation by nurses and other non-laboratory personnel.

Self-monitoring of blood glucose is a well established and generally accepted method of improving diabetic control and minimising long term complications. The different reagent strips on the market are all similar in principle and apart from timing differences and washing or wiping of the strips each involves comparing a reacted strip at a fixed time either visually against a calibrated colour scale or electronically with a meter. Both systems have points for and against. Briefly, visually read strips do not require financial outlay for, or maintenance of, a meter but they can be affected by different light conditions or differing interpretation of colour. It is worth noting that diabetics are prone to visual impairment. Meters involve an outlay of around $270 but when properly operated are more accurate than visually read strips. Both systems are only as good as the person using them and, with the exception of the glucometer with a normal range control, lack any quality control. We have numerous examples of patients going for long periods faithfully maintaining records of their daily glucose levels measured with their meters and discovering, when they are checked by our laboratory, that they are producing totally erroneous results.

PATIENT (hypocount meter)	LABORATORY
12.9	17.9
16.8	35.0
20.1	29.5
16.9	25.4
16.9	26.7

GLUCOSE MMOL/L

Table 1

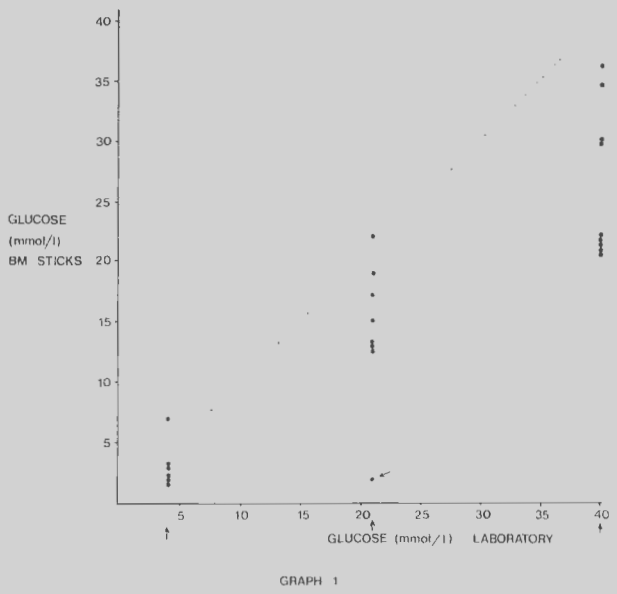
Doctors order a glucose meter on behalf of their patient or prescribe BM sticks and send the patient away to monitor their glucose with neither training or instructions. They fail to realise that the Glucometer is basically a portable laboratory instrument, albeit simple, and that it requires training and practice to become proficient. Practice is also essential for BM sticks. In one instance a G.P. issued an elderly woman with a packet of Visidex (presumably left by the sales rep) and a syringe needle and told her to measure her glucose. She arrived at the hospital diabetic clinic totally confused and very upset. It is clear that some doctors do not appreciate the efforts made by a laboratory to quality control results and endow figures produced by a patient or a laboratory with equal importance.

The most frustrating aspect of this problem from a technologists point of view is the lack of quality control and the realisation that patients are not getting the full benefit of the systems available, that in many cases their management is suffering, and being able to do very little about it. Ideally there should be a record of all patients owning meters or using strips and a regular quality control and (for meters) maintenance program could then be organised. In Nelson and presumably in other centres, there is no way of knowing who has meters when they are ordered through G.P.'s. The patients whose meters are ordered through the diabetic O.P. department do have the meters and necessary quality control explained and informed that they can visit the laboratory at any stage to have their meters checked. In spite of this very few do come to the laboratory although some bring their meters to the diabetic O.P. to be checked against the reputedly reference meters used there.

In response to these problems we now offer a system of measuring glucose on dried blood spots impregnated in filter paper. The method is a modification of one described by Albyholm in 1981[1]. Blood is spotted onto borate treated filter paper. When dry, discs are punched out and eluted with perchloric acid. An aliquot of the eluate is assayed using a glucose oxidase method and a correction factor to compensate for disc size and dilution is applied to the result. The correction factor was derived by assaying 35 samples by this method and our laboratory's routine method. Correlation with our routine method is excellent: $y = 1.01x - 0.10$, $r^2 = 0.977$, $n = 32$. and cost per test is fifteen cents. We supply a kit with space for eight tests, eight lancets and an instruction sheet. These kits offer diabetics an alternative to using BM sticks or purchasing a meter or for those patients who already have these systems it offers an easy Q.C. program. Diabetics using the system to monitor strip/meter performance simply soak one circle of the filter paper with a drop of blood at the same time they use their strips or meter, and note the time and date details on the card. When the card is full, or sooner if required, it is delivered to the laboratory where it will be processed the day it is received. The patient includes their results and the laboratory contacts them if there is a significant discrepancy. For those patients without strips or meters the system is similar, and results are sent to them and to their G.P. The system is also ideal for people who do not routinely use a meter or strips e.g. pregnant diabetics and patients in outlying areas who need not interrupt their daily routine to attend the laboratory for a sugar series. Our system is still in its infancy and has not been widely advertised but at this stage appears very satisfactory.

Ward based blood glucose testing is a different situation. The advantages of the BM stick or meter to the clinician include speed of obtaining results and the absence of form-filling. The disadvantages are much less obvious to clinicians but are apparent to laboratory staff. We are in no doubt, from our experience in training nurses to use BM sticks, that they are far from suited to the ward situation. In training situations, levels on spiked bloods were usually grossly underestimated and the following results were obtained from one ward on a visit to check the performance of "trained" nurses.

Comments from nurses such as "you've usually got an idea what the patient's blood glucose is anyway" do not inspire confidence. From our observations, house-surgeons are even more of a risk to the patients than nurses when using BM sticks as they have had no training or practice on blood with known values. Examples of their "expertise" include a patient diagnosed as "hypo" with a glucose of 1 mmol/l subsequently measured by the laboratory at 8 mmol/l., and a patient in ketoacidosis with a glucose measured at 30 mmol/l by BM stix which was actually



Graph 1: Shows the spread of results obtained by nurses using BM sticks at 3 concentrations of glucose.

greater than 60 mmol/l. After prolonged protest from the laboratory a set of guidelines in the use of BM strips was established and it was agreed that the primary use of the strips was the monitoring of patients on total parenteral nutrition to ensure steady carbohydrate load. The other use was to be in conjunction with the laboratory for training new diabetics. This protocol is regularly being ignored and sticks are still being used in all situations including management of ketoacidosis patients. It is interesting to note from graph 1, that a glucose of 21.0 mmol/l was measured by one nurse as 22.0 mmol/l and another as 2.0 mmol/l. Presumably if nurse A did a glucose before going off shift and reported 22.0 mmol/l and nurse B did one after coming on duty and measured it at 2.0 mmol/l, the patient would be treated when in fact the glucose was elevated but stable. Another aspect is the cost. At the present rate of use in the wards 6,500 tests will be done, at a cost of around $2,600 this year. Most of these will be completely unnecessary and a lot of them will be done during normal laboratory working hours.

The nominal BM stick range of up to 44 mmol/l gives a false sense of security in that an ability to interpolate betwen two colours representing levels of 22 and 44 is assumed. As mentioned earlier, such attempts at interpolation are made even when the colour ought to be recognised as greater than 44. We do not believe that a keto-acidosis can be reliably assessed or efficiently treated using data obtained from such operator-dependent procedures.

In retrospect, a meter (assay range limited to levels of 22 mmol/l and below) with the use restricted to authorised nursing staff would have been a better system to introduce for any essential ward tests. This would allow for full documentation of times and results of tests and the system could be audited by the regular issuing of a control blood by the laboratory.

We are very concerned and not a little frustrated that our experience and expertise is apparently set at nought by medical staff who seem more anxious to have an instantaneous figure than to ensure that the figure accurately reflects the patient's status.

There is considerable inconsistency in having medical laboratories working towards TELARC registration and at the same time allowing the non-laboratory use of uncontrolled analytical systems in a critical health care situation.

References:

1. Abyholm A.S. Determination of glucose in dried filter paper blood spots. *Scand. J. Clin. Lab. Invest.* 1981; **41:** 269-274.

Some Thoughts on Reference Ranges

Peter D. Hill

Dept. of Mathematics, University of Waikato, Hamilton

Presented at the Association of Clinical Biochemists Conference, Hamilton, August 1983.

Abstract:

Well-known conceptual and definitional difficulties for reference ranges are viewed and recommendations are made. Also discussed is a particular area of controversy between the Gaussian approach, mean ± 2 standard deviations, and the percentile approach to constructing reference ranges. A judgement on that controversy is made in the light of a recent computational study. The remaining sections set out some alternative concepts to reference ranges. They make use of the new powers of data analysis made available by current computer technology. These techniques represent different approaches by which the laboratory scientist might assist the clinician in diagnosis.

Introduction:

My introduction to the subject of reference ranges was a series of questions from Waikato Hospital laboratory staff. The questions were of the following sort. Consider creatinine as an indicator of renal dysfunction. Among healthy patients the range of values may have a distribution as in Figure 1 (a), whereas when the kidney is not functioning properly the distribution of creatinine values has a higher mean (and in Figure 1 (a) it has been drawn with a greater dispersion).

In a hypothetical data base of individuals we may get a distribution of sample values as in Figure 1(b). There appear to be two "humps" or modes. Is this a genuine feature of creatinine values among the healthy population, or more likely, does the second hump represent a group of people who have renal dysfunction and have not been screened out from the healthy people? Or are they people who cannot be screened out because their disease is as yet sub-clinical? Other possible outcomes from a sample of individuals are shown in Figures 1(c) and (d) where there is a long right tail to the distribution of values. Again, does this represent an inadequate screening out of unhealthy people or is it a genuine feature of healthy creatinine values? Or perhaps the outlying values might be spurious mismeasurements or misrecordings.

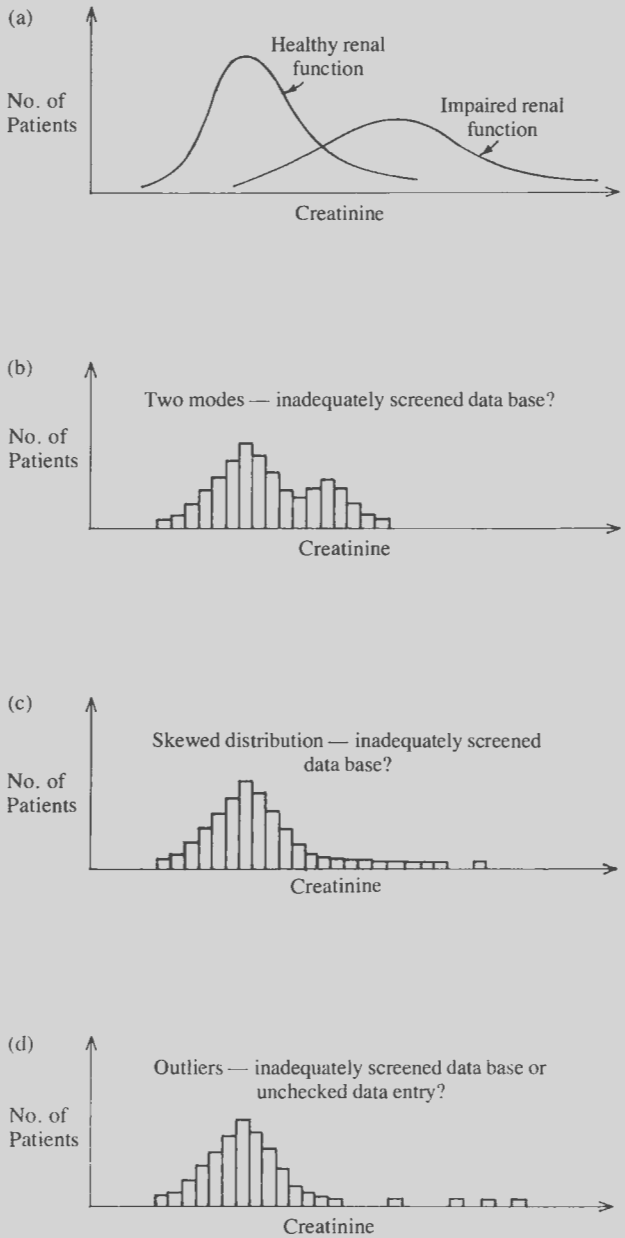
These deceptively simple questions illustrate the difficulties associated with the construction and use of a reference range, which is essentially a rather simple statistical device. The comments below shed some light on these questions and others at a similarly basic level.

Definitions, Deficiencies and Difficulties

What is a reference range? The following quotations attempt to define and describe its purpose.

"A reference range is defined by those points which on the average will include 95% of the population and are obtained by testing an adequate number of clinically normal subjects on the assumption that the values are from the same stable population."[1]

Figure 1
Hypothetical Creatinine Values



"The purpose of the normal biochemical range for any one blood constituent is to describe as accurately as possible the limits inside which most healthy people fall, and to help the physician establish whether a patient is in a healthy or diseased state."[2]

"A single range defined simply as containing a large fraction of healthy readings may be of value to clinicians in spite of the fact that its limits are not optimal boundary values for any specific diagnostic or therapeutic decisions."[3]

An important emphasis in these definitions is that measurements are made on healthy individuals. This may be difficult to achieve in practice and some authors have asserted it is unnecessary in principle as well, but I believe it is important in principle. The reference range is a simpleminded, blunt instrument of diagnosis. In relation to creatinine as an indicator of renal dysfunction, for example, the reference range simply says that among healthy individuals 95% have a creatinine level within the range. Anyone above the top end of the range is considered to be showing signs of renal disease. By definition 2½% of healthy people have levels above the top end of the range; that is the false positive rate. Because we have no information on the diseased population the reference range gives no control over, or measure of, the rate of false negatives. They are the sufferers from renal disease who happen to have creatinine levels below the upper point of the healthy range. Some authors, for example Hoffman[4], have advocated using the hospital population for constructing reference ranges without consideration of whether they are healthy or not. This misses the basic definition point of reference ranges. If they are not based on healthy patients we have no knowledge of the false negative rate **or** the false positive rate. The definition of a state of health is a notoriously vexed question. Several articles in Grasbeck and Alstrom's[5] Proceedings has led to a formal recommendation from the International Federation of Clinical Chemistry (IFCC) on definitions of basic terms and concepts underlying the construction of reference ranges.

This issue of the need for a healthy data base is discussed further below, but at present it raises two other issues. First is the familiar problem of the plethora of meanings of the word "normal". Alstrom[6] summarizes 7 different meanings identified by Murphy[7]. Perhaps the most obvious confusion in this context is between normal, meaning healthy, and normal, being the name of a statistical distribution, now more generally called Gaussian to avoid the confusion. Feinstein[8] suggested the term customary range in place of normal range. However, IFCC has recommended the term reference range which appears to be winning widespread acceptance.

The other issue which arises at once is the epidemiological population to which the reference range is intended to refer[8]. This should be clearly understood and if feasible clearly conveyed to the user of the range. There is now a general appreciation of the need for age and sex specific ranges for many substances. This information too should be clearly conveyed to the clinician. Age and sex specific ranges can give rise to their own practical and conceptual difficulties. Often it is hard to get a usefully large data base in the various age categories, particularly at the extremes of the age range. Or consider high blood pressure — a recording which is well within the reference range for 70 year old males may be well outside the general reference range for the whole population. How should we interpret age-specific ranges in such a context? There may be a need for ranges specific to particular ethnic groups, although these too suffer from the above conceptual difficulties.

The routine reference range contains 95% of healthy individuals. It is now generally appreciated that this is merely a conventional percentage. It may be that sometimes a 90% range would be a helpful warning device to the clinician. Of course, there could be some confusion when a list of ranges is returned on a laboratory report if some were 90% ranges, some 95%, some 99%. Obviously, the content of a range would have to be clearly stated.

Similarly the two-sidedness of reference ranges is also only conventional. In a 95% range 2½% are deemed to have unusually low values and 2½% unusually high values. For many substances high values are of concern to the clinician rather than low ones. Low bilirubin values, for example, are not associated with any particular disease condition, so to cut off the bottom 2½% of values has no diagnostic relevance. I believe one-sided ranges should be more commonly used — again with a clear explanation to the user.

There are several much more intractable problems. A reference range mainly concerns variability between persons but it also includes analytic (measurement) variability. And of course there are other sources of variability, within a person over time, between laboratories etc. Stamin[9] gives references to many articles suggesting strategies for dealing with the various different sources of variability. The least that can be said is that the clinician ought to be made aware of at least the analytic variability of the various recordings on a laboratory report.

The between laboratory variability also speaks strongly in favour of local rather than national or international norms. So too does the need to be concerned with the specific local epidemiological population.

The worst difficulties relate to repeated recordings on an individual and the interpretation of several related measurements at once. The reference range is not a suitable tool for the latter situation. As to the interpretation of repeated recordings over time, it can only be said that the clinician should be cautious, appreciating that the probability content of repeated 95% ranges does not remain at 95% but is modified in an uncontrolled way.

A Resolution of a Technical Controversy

We turn now to a particular well-known controversy on the technical side; namely whether reference ranges should be constructed using the Gaussian assumption or by the percentile approach. If it is assumed that the distribution of values of a substance has Normal (or Gaussian) shape, this assumption gives rise to the standard form for a 95% two-sided reference range, mean ± 2 standard deviations. But it has been well-known, for many decades, that many, if not most, biomedical

variables do not follow a Gaussian distribution. There is commonly a tendency for biomedical variables to have a long right tail of values. But there are other frequent departures from the bell-shape symmetry of the Gaussian distribution — for example two or more humps or modes, or an excessive sharpness or flatness of the bell-shape. The ghost of Gauss has been exorcised several times[8,10] from the domain of reference ranges but why was it ever invoked in the first place? The reasons are partly historical. The statistical theory of normal range was developed by Wilks and others 40 years ago in the context of industrial quality control. In that field the Gaussian distribution may be widely appropriate. Another argument in favour of the mean ±2 standard deviations points out that the long right tail which characterizes many distributions in the medical field may be drawn in to give a semblance of a Gaussian distribution by dealing with the logarithm of the values.

In 1958, Herrera[11] made generally known the percentile technique for constructing reference ranges. This method assumes no particular form for the population distribution of values. In simple terms it says that the central 95% of a distribution of values may be estimated simply by the central 95% of the sample values. So the population 97½ percentile, that is the value below which 97½% of healthy people's values lie, is estimated by that value in the sample below which 97½% of the sample values lie. The percentile technique is attractively straight forward and free of assumptions. Naturally it has been enthusiastically advocated in the literature. It is a valid statistical method put forward by Wilks in 1941 in the same paper[12] in which he introduced the mean ±2 standard deviations range. However, the method has a significant weakness in that it is much less reliable than the Normal assumption method. This is because the end points it quotes for a reference range depend crucially on single values in the data base whereas the Normal-assumption range uses **all** the data in calculating the mean and standard deviation. So the percentile method pays for its freedom from assumptions on the form of the distribution of values by being inherently more variable.

The problem of variability in the actual attained content of reference ranges is a complicated one. Suffice to say that a 95% range contains 95% of healthy individuals **on average** i.e. averaged over repeated applications of the process of constructing a range. As an acknowledgement of this inherent variability I recommend that a quoted reference range invariably carries with it a statement of how many individuals it was based on. Obviously the larger the data base the more reliable the range.

The inevitable consequence of this variability of the percentile technique was a backlash against it. Several authors, for example Harris and De Mets[13], have developed quite sophisticated ideas for transforming non-Gaussian data to something closer to Gaussian so that the mean ±2 standard deviations can still reasonably be used. I suspect the net result of this controversy has been some uncertainty and confusion for those who construct reference ranges. It is not unknown for a laboratory to routinely calculate both the mean ±2 standard deviations **and** the percentile-type range. The two are then mixed in an ad hoc way to produce the published range. That is an understandable but less than optimal response to a confused situation.

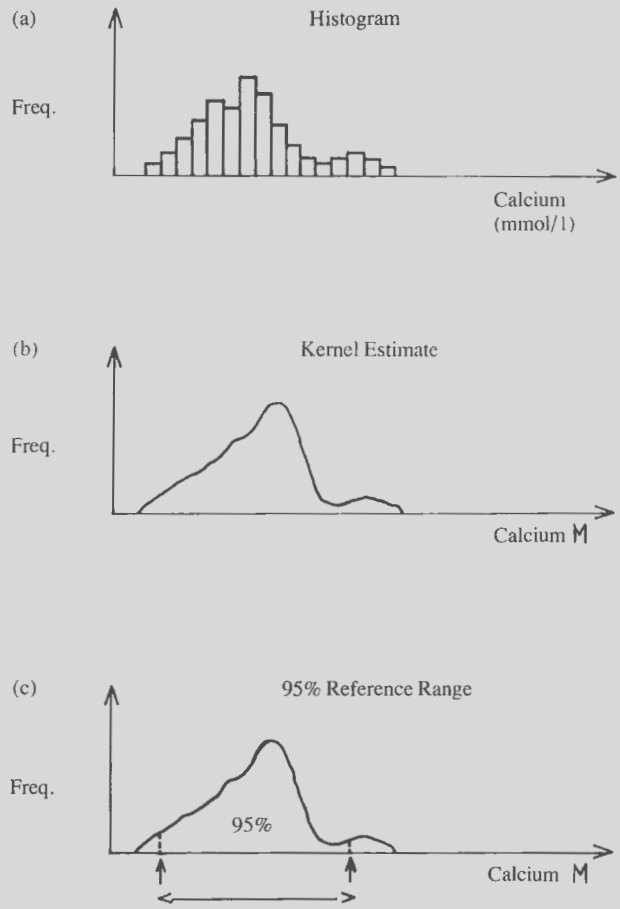
Recently with a colleague I set out to compare the two techniques by means of a computer simulation study. We generated artificial data from a computer's data generation capabilities to simulate a variety of shapes of distribution and we compared how the two methods fared under these various conditions. The conclusion of Chan and Hill[14] is as follows:

"A histogram (frequency bar chart) for the sample data is a necessary preliminary as it provides a good indication of the shape of the distribution of values. If the data appear Normally distributed (symmetric, bell-shaped) or approximately so, or are moderately skewed to the right, the mean ±2 standard deviations range is superior to the percentile range, particularly when the date base is small (n< 100). If the data exhibit bi-modality (two humps) or some other features of marked non-Normality, the percentile method will be better but a larger sample size (n> 100) may be required."

The Kernel Estimation Technique

I do not believe that sophisticated transformations to induce a Gaussian distribution are justified in this context — Harris and De Mets[13] have even advocated inverse arc sine transformations! There is now though a middle ground between the Gaussian assumption and the percentile approach. This depends on a new statistical methodology known as kernel estimation of distributions. It is not possible to describe the details here but the aim is clear. If we are not willing to assume a particular form, notably Gaussian, for the distribution of the recordings, we are left with the data itself and a graphical representation, say the frequency bar chart. But we should feel uncomfortable about relying too heavily on the data **alone** because they are just the recordings on a random sample of healthy individuals. Another sample will give different recordings. Without any assumptions at all we may be a little too much at the whim of the particular data. The kernel estimation concept takes us, in a sense, midway between the Gaussian distribution assumption and the data just on its own. For example, suppose we had 100 serum calcium recordings and that their histogram (or frequency bar chart) looked as in Figure 2 (a). Relying solely on the data with no assumptions on the form of the distribution this is all we have and we could apply the percentile method to obtain a reference range. But the kernel method gives a better result, still with no other assumptions on the data.

Figure 2



The kernel estimate of the distribution of values is like a smoothed version of the sample histogram. It takes account of the sampling variability and smooths the distribution of values until a smooth curve is obtained, as in Figure 2(b), rather than just spikes at the actual data values.

The kernel reference range is then found by identifying (by mathematical techniques of integration) the points on the kernel graph between which lie the required percentage of the distribution of values, as in Figure 2(c).

In Hill[15], I have shown that the kernel approach does in fact slightly reduce the inherent variability in the percentile approach and give rise to more reliable reference ranges and yet without the need to make any assumption on the distribution of the values. However, the main benefit of the kernel approach results from the graphical picture obtained of the distribution of values — we return to consider that benefit below.

Quoting a Patient's Percentile Value

An alternative mode of thinking about the reference range concept has come from Elveback[16] and others at the Mayo Clinic. Suppose that a reference range for serum inorganic phosphorus is 0.8 to 1.4 mmol/l.

and early 1960s, using paper and ion-exchange chromatography, resulted in the discovery of numerous aminoacidopathies. Many countries now carry out mass screening of neonates for some of these aminoacidopathies using blood or urine, by either specific microbiological inhibition assays[9,10] for individual diseases with a relatively high incidence e.g. phenylketonuria, or more comprehensive chromatographic procedures that can detect at least 18 of the known aminoacidopathies[6,7,11-16]. In all cases, the inborn errors detected by these methods are limited to those with defective enzymes in the first two steps of amino acid metabolism, that is, they are all dependent on the accumulation of the parent amino acid or its keto metabolite[17]. Other inborn errors, resulting from impairment of enzymes in the later stages of amino acid metabolism, are associated with the production of characteristic "organic acids" with or without an abnormal aminoacidaemia or aminoaciduria. These disorders frequently present with an acute metabolic illness in early life[18].

An important consequence of the intensive investigation of the organic acid disorders has been the realisation that many of the patients who have been described do not have a primary impairment of an enzyme associated with amino acid degradation, but have a defective synthesis of the co-enzyme required for the normal activity of that enzyme. Once such a defect is recognised in an organic acid disorder, there is often the possibility of treatment by co-enzyme therapy: early diagnosis is, therefore, essential.

Organic Acids and the Organic Acidaemias

The term organic acid has generally been used to describe water-soluble carboxylic acids, with or without keto or hydroxyl groups. Short-chain fatty acids, di and tricarboxylic acids, hydroxy and keto acids are included in this category, but amino acids are usually excluded. Therefore, the chemical characteristics that are common to organic acids are water solubility, acidity and a negative ninhydrin reaction. Many of the organic acids originate from the metabolism of amino acids; however, some organic acids, for example propionic and pyruvic acids, are partly produced from other sources, such as lipids or carbohyrdates.

The organic acidaemias comprise a group of inborn errors of metabolism characterised, not by an amino acidaemia or amino aciduria, but by the accumulation of organic acids due primarily to an inherited defect or the specific inhibition of the enzyme(s) responsible for the further metabolism of the pertinent organic acid(s). In some disorders of organic acid metabolism, accumulation of abnormal organic acid may be found in urine but not in blood. Such diseases should be called organic acidurias; however they are included as organic acidaemias for convenience of discussion.

The technical limitations of conventional paper and ion-exchange chromatography made the detection of organic acids difficult. The development of gas chromatography (GC) and mass spectrometry in conjunction with gas chromatography (GC-MS) led to the discovery, in 1966, of isovaleric acidaemia, an inborn error of leucine metabolism, by Tanaka et al[19]. Within a few years, two more diseases, methylmalonic[20,21] and propionic acidaemia[22] were found by the use of similar methods. Numerous other organic acidaemias have been found in recent years, and knowledge of these is becoming increasingly important in paediatrics and medical genetics. Information on nomenclature, metabolic block(s), clinical presentation and abnormal urinary organic acids is shown in Table I.

There are several common features among the organic acidaemias. First, they have been discovered and chemically characterised primary by GC and GC-MS. These inborn errors are not detectable by paper and ion-exchange chromatography of amino acids, since the major substances that accumulate are not the parent amino acid but rather its metabolites. The second important feature of organic acidaemias is that the major abnormal metabolites that accumulate may not be the utilized substrate of the defective enzyme, but may be the product(s) of the substrate modified by alternate pathway(s). The unutilized substrate may, or may not, accumulate in body fluids. Isovalerylglycine and β-hydroxy-isovaleric acid are the major urinary metabolites in isovaleric acidaemia, whereas the unaltered substrate, isovaleric acid, accumulates in blood only episodically[23,24,25]. Urinary excretion of isovaleric acid is very small, even when it accumulates in blood[25]. Similar observations have been made in β-methylcrotonyl CoA carboxylase deficiency[26,27] and in propionic acidaemia[28,29]. Two additional alternate pathways have been observed in propionic acidaemia. These are the synthesis of long-chain fatty acids with odd numbered carbons[22], and methylcitrate synthesis[30]. Knowledge of these alternate pathways is important, in order that the correct diagnosis can be

Table I
Organic Acidaemias: Nomenclature, Metabolic Defects, Clinical Presentation and Abnormal Urinary Organic Acids

Disease	Metabolic Defect	Clinical Presentation	Abnormal Urine Organic Acids
Isovaleric acidaemia	Isovaleryl-CoA dehydrogenase	Acidosis, seizures and odour of "sweaty" feet in infancy or more chronic course with episodes of vomiting, acidosis, lethargy and typical odour.	Isovalerylglycine and β-hydroxyisovaleric acid
3-methylcrotonylglycinaemia	3-methylcrotonyl-CoA carboxylase	Very variable; acidosis, feeding problems and odour of "cat's urine" fairly consistent	3-methylcrotonylglycine and β-hydroxyisovaleric acid
3-Hydroxy-3-methylglutaric acidaemia	Hydroxymethylglutaryl-CoA lyase	Acidosis and hypoglycaemia without ketosis in newborn or during first year	3-Hydroxy-3-methylglutaric, 3-methylglutaconic, 3-methylglutaric and 3-hydroxyisovaleric acids
2-methyl-3-hydroxybutyric acidaemia	Beta-ketothiolase	Ketotic hyperglycinaemia syndrome in infancy or chronic course with episodes of vomiting, acidosis, hepatomegaly and encephalopathy	2-methyl-3-hydroxybutyric and 2-methylacetoacetic acids; tigylglycine often present
Propionic acidaemia	Propionyl-CoA carboxylase	Ketotic hyperglycinaemia syndrome in newborn or later, hyperammonaemia common, occasionally asymptomatic	Methylcitric and β-hydroxypropionic acids
Methylmalonic acidaemia	(a) Methylmalonyl-CoA carbonylmutase (b) Biosynthesis of adenosyl-B_{12}	Same as propionic acidaemia Features of CNS dysfunction and megaloblastosis may be present when defect in B_{12} coenzyme biosynthesis is early	Methylmalonic acid
Glutaric acidaemia	Glutaryl-CoA dehydrogenase	Progressive extrapyramidal movement disorder in childhood ± episodic acidosis, vomiting and encephalopathy	Glutaric acid consistent, 3-hydroxyglutaric acid usual, and glutaconic acid occasional (usual during ketosis)
Pyroglutamic acidaemia	Glutathione synthetase	Acutely in infancy with acidosis and haemolytic anaemia	Pyroglutamic acid
D-Glyceric acidaemia	(?) D-Glyceric dehydrogenase	Mental retardation, seizures, hypotonia and acidosis	D-Glyceric acid

made and for a better understanding of the clinical symptoms (as well as the absence of symptoms during periods of remission). Thirdly, some biochemical changes not directly related to the primary enzyme defect have been observed in several organic acidaemias. These are shown in Table II. The accompanying biochemical changes can be misleading if too much emphasis is placed on them, while the primary biochemical defect is overlooked.

Clinically, all of the organic acidaemias are accompanied by severe metabolic acidosis, either persistent or intermittent, with recurrent vomiting, lethargy and coma. Patients often have an unusual smell (the two patients described by Tanaka et al[19] were diagnosed because of an offensive smell in their breath, urine and sweat, described as the smell of 'sweaty feet'). The clinical pictures of the diseases in this group are so similar that it is not possible to make a diagnosis merely from clinical symptoms. Diagnosis must be made by chemical analysis and enzymatic assay.

Organic acidaemias are not only secondary to genetic enzyme defects, but may also be induced by nutritional and toxic factors. An example of induced organic acidaemia is the methylmalonic acidaemia of Vitamin B^{12} deficiency[31], which was known several years before the discovery of the genetic methylmalonic acidaemia. However, the major symptoms are secondary to the haematological and neurological manifestations of Vitamin B^{12} deficiency, rather than to the methylmalonic acidaemia itself. The vomiting sickness of Jamaica is caused by ingestion of the unripe ackee fruit which contains the toxin hypoglycin A. Isovaleric acidaemic and hypoglycin A intoxication are examples of an identical organic acidaemia, in one case due to an inborn error, and in the other due to a toxin[25].

Table II
Secondary Biochemical Changes in Organic Acidaemias

Secondary biochemical changes	Primary inborn errors
Hyperglycinaemia	Methylmalonic acidaemia Propionic acidaemia α-Methylacetoacetyl CoA-β-ketothiolase deficiency Isovaleric acidaemia*
Hypoglycaemia	Methylmalonic acidaemia Propionic acidaemia
Hyperammonaemia	Methylmalonic acidaemia Propionic acidaemia α-Methylacetoacetyl CoA-β-ketothiolase deficiency
Long-chain ketonuria	Methylmalonic acidaemia Propionic acidaemia α-Methylacetoacetyl CoA-β-ketothiolase deficiency
Odd-number carbon fatty acids	Propionic acidaemia

* Slight hyperglycinaemia in a single case

Collection, Storage and Transportation of Specimens

Although increasing in number, there are still few clinical laboratories and hospitals equipped to undertake the complex task of organic acid profiling. Transportation of clinical specimens is necessary when clinicians have to rely on specialized laboratories for carrying out the complete chromatographic profiling. It is important that the samples should undergo as little change as possible from the time of collection until analyzed.

Recommendations for the collection, storage and transportation of physiological fluids for chromatographic profiling have been made[32]. Blood samples from fasting patients and urine collected in the morning, without preservatives, appear to be preferred. Both urine and serum samples should be frozen and stored at -20°C or lower temperatures, so as to prevent decomposition of metabolites. When morning urine samples are analyzed, quantitative results are usually expressed relative to the creatinine concentration. For more exact quantitative data, 24 hour urine samples should be collected. It is important that information concerning drug intake or dietary habits should be obtained[33-36]. The presence of drugs or drug metabolites are probably the major cause of unusual peaks noted on gas chromatograms[32].

Isolation of Organic Acids from Protein-Containing Body Fluids

Urine is the only human body fluid that is normally devoid of proteins. All other body fluids require treatment to remove the varying amounts of protein they contain. Several methods may be used to remove protein; all have their advantages and disadantages.

If non-destructive methods, for example dialysis, gel and membrane filtration, are used to remove proteins prior to GC analysis, the tightly protein-bound and/or water soluble, low molecular weight organic acids, such as long-chain fatty acids, will be lost. Non-denaturing methods should be used with caution if the aim is to achieve a total organic acid profile.

Protein precipitation with, for example, sulphosalicylic acid followed by extraction of the organic acids with organic solvents, may be used although certain organic acids may be co-precipitated with the denatured protein.

A convenient method, widely used for obtaining profiles of total organic acids in protein-containing fluids and tissue homogenates, uses cold ethanol as a precipitating agent[37-39]. After removal of the proteins by centrifugation and removal of the ethanol in vacuo, the resulting aqueous solution can be treated as if it were urine.

Isolation of Organic Acids from Urine and Deproteinized Body Fluids

Three principal methods for the isolation of organic acids from urine and deproteinized fluids, prior to derivatization and GC, are currently in use. The first is based on solvent extraction, usually with diethyl ether and/or ethyl acetate. Extraction using manual or mechanical shaking is normally carried out, although these procedures are not quantitative[40]. More quantitative extraction is obtained by continuous extraction overnight. The second method is based on an ion exchange[41] and is used in many laboratories. Ion-exchange methods give quantitative recoveries of the organic acids. DEAE-Sephadex is the most widely used ion-exchanger although other resins, for example Dowex 3, are also suitable[42]. After retention on the resin, the acids are usually eluted with aqueous pyridinium acetate[33,41] before lyophilization, although, in order to avoid any loss of the more volatile acids during the lyophilization step, elution with hydrochloric acid, followed by neutralization of the eluate with sodium hydrogen carbonate before lyophilization has been suggested[43]. A third, more specialised, method, limited to the isolation of the more volatile constituents, makes use of either steam distillation[19,22,44] or vacuum distillation[45], to isolate the organic acids from biological material.

Each of the methods described has its place in the isolation of organic acids. In general, for those disorders characterized by gross metabolic changes, the solvent extraction system is applicable. This is especially important in emergency cases, for example on sample from severely ill, acidotic children, where a rapid result is required. For quantitative work[33-35] and work involving subtle changes in the organic acid profiles, the use of anion-exchange and distillation procedures is essential. Therefore, those laboratories engaged in definitive diagnosis generally use both quantitative and qualitative procedures.

Derivatization of Organic Acids

Gas chromatographic procedures can only detect volatile constituents and compounds that can be converted into volatile derivatives. The organic fraction of biological fluids contains mono- and polycarboxylic acids, mono- and polyhydroxy acids, keto and phenolic acids, phenols and conjugates of the organic acids, particularly with glycine. There have been numerous publications dealing with methods for the preparation of volatile derivatives of the organic acids; all are based on silylation and methylation.

Trimethylsilyl (TMS) ethers and esters are probably the most popular derivatives used for studying the organic acids. TMS derivatives are comparatively easy to prepare, safe to handle and most of them have excellent chromatographic properties. The libraries of mass spectral data[46,47] contain more information on TMS derivatives than any other type of derivative. The most widely used silylating reagents are bis (trimethylsilyl) trifluoroacetamide (BSTFA) and bis (trimethylsilyl) acetamide (BSA) which form derivatives with the carboxyl, hydroxyl and phenol groups of organic acids. Certain metabolites, particularly keto acids, have a tendency to yield multiple derivatives and there are problems, also, with the silylation of short-chain dicarboxylic acids[42], and o-hydroxyhippuric acid (salicyluric acid)[48]. In these cases, methylation appears to be the preferred method. Other workers have used oxime-TMS derivatives[49], methoxime-TMS derivatives[50] and ethoxime-TMS derivatives[33], although the latter authors prefer ethoxime-TMS derivatives for the determination of urinary organic acids in general[33-35].

The methylated esters of organic acids also have excellent chromatographic properties and can readily be prepared. The mass

spectra of methyl esters are usually simple and are often easier to interpret than those of the corresponding TMS derivatives. It is usually easier to predict the fragmentation pattern of a methyl ester than that of the silyl ester, which is particularly useful when searching for the presence, in a body fluid, of a given metabolite, even if the authentic compound and/or information on its GC-MS behaviour are lacking. It is for this reason that methylation techniques are frequently used in preference to silylation techniques[37,51-53]. Diazomethane is the preferred methylating reagent, despite the hazards associated with its use (diazomethane is a known carcinogen). This method, like silylating procedures, may also lead to certain by-products. For example, more than one derivative is usually formed by the action of diazomethane on keto acids, and an artifact resembling 3-hydroxypropionic acid is formed if water is present during methylation[54]. Diazomethane will methylate the hydroxyl groups of phenols and the thiol group of thiols, as well as the carboxyl groups. Thus a number of important phenolic acids for example p-hydroxyphenyllactic acid, may yield one or two derivatives depending on methylation time and temperature.

Alternative methylation procedures include esterification with methanol/hydrochloric acid, methanol/thionyl chloride or methanol/boron trifluoride and also on-column methylation[55]. In the latter method, the organic acids are converted into their trimethylanilinium salts by the addition of trimethylanilinium hydroxide and subsequent pyrolysis of the salts in the injection port of the GC generates the methyl esters[55,56].

The relative merits of the various approaches considered are frequently discussed. There is no simple answer, as all methods have their advantages and disadvantages. The main point, and the point Stokke[57] made is, to become familiar with one method, to learn its pitfalls, and to be able to use alternative procedures when required.

Techniques Used in the Measurement of Organic Acids

Preliminary screening for organic acids may be done by paper or thinlayer chromatography (TLC)[58,59] except for the volatile fatty acids. Because of the low resolving power of these methods, their use should be limited only to screening. Paper and TLC should not be used for identification, because a series of homologous compounds would give similar Rf's. By these methods, for instance, various acyl glycines — such as isovalerylglycine[23], methylcrotonyl glycine[26,27,60], propionylglycine[28] and benzoylglycine (hippuric acid) are not distinctly separated from each other. The main point of emphasis is that there are a vast number of organic acids in human body fluids, and identification must rely on more advanced analytical techniques.

Most workers have favoured the use of gas chromatography (GC) for separation purposes, combined with mass spectrometry (MS) to identify the individual components in the body fluids. Gas chromatography is a procedure in which the volatile components of a mixture are separated by partitioning between a moving, inert carrier gas and a non-volatile, immobile liquid (the stationary phase)[61]. The latter is contained in a column and is coated onto an inert, sizegraded solid (the stationary support). The carrier gas is passed through the column as column temperature is raised and as components of the mixture leave the stationary phase they are carried to a detector and become recorded as a series of peaks.

The technique of mass spectrometry rests on the observation that an ion radical is created when a compound is vaporized and when exposed to an electron beam in a near vacuum, will disintegrate in a manner absolutely characteristic of its parent compound[62]. The array of small fragments that is created constitutes the mass spectrum; the mass spectrometer creates these fragments and records their weight and relative abundance as they are impacted onto a detector. The modern mass spectrometer can generate a spectrum every two or three seconds, which is fast enough to "catch" individual compounds as they are eluted from a GC. The visual comparison of unknown spectra with a catalogue of known mass spectra often gives valuable clues with regard to the structure of the unknown. Manual interpretation of the mass spectra generated is, however, often difficult and time consuming. Various groups have utilized both on-line and off-line computer systems to identify the mass spectra generated[63,64]. Whilst initially designed to measure a very small number of components in any one mixture, called Selected Ion Monitoring, laboratories using GC-MS Computer systems have generally developed their systems to perform repetitive scanning of the full mass range, followed by analysis of selected spectra[37,41,50,65].

Gas chromatography can detect only volatile compounds or volatile derivatives. Because of this limitation, it has been estimated that gas phase analytical methods can only detect about 20 percent of the total number of substances present in complex biological materials[37,39]. High performance liquid chromatography (HPLC)[66-69] and HPLC coupled to mass spectrometry[70-72], rapid scanning fluorescent spectroscopy[73] and ion chromatography[74], are techniques which may yet prove useful, for the analysis of organic acids. Gas chromatography and GC-MS remain, at the present time, the major analytical techniques for the analysis of organic acids.

Prenatal Diagnosis of the Organic Acidaemias

It should be possible to perform in utero diagnosis of organic acidaemias based on ezyme studies in cultured amniotic cells. To date, however, only fetuses with methylmalonic acidaemia, propionic acidaemia and glutaric acidaemia have been diagnosed prenatally[75-79]. Changes in amniotic fluid organic acids have been demonstrated in fetuses affected by these three conditions, and with pyroglutamic acidaemia[76-81]. There can be no doubt that prenatal diagnosis of the other known organic acidaemias based on fibroblast enzyme studies has considerable potential. Improvements in chromatographic instrumentation resulted in the improved understanding, and further discoveries of disorders of organic acid metabolism in the 1960's and early 1970's; it does not seem improbable to speculate therefore that those same organic acidaemias will be detectable prenatally with the future development of more sensitive fibroblast enzyme assay techniques.

Conclusion

The organic acidaemias have been the subject of intensive research since the discovery, in 1966, of isovaleric acidaemia[19]. Advances in gas phase analytical techniques, especially as they relate to column technology and improvements in computer methodology have opened up new possibilities, not only for the study of gross alterations in organic acid profiles, but also for the detection of more subtle changes. Comprehensive screening programmes, designed to detect metabolites including organic acids, and based on these gas phase analytical techniques, are preferred to the traditional, yet slower, paper and ion-exchange methodologies. The analysis of organic acids is a time-consuming process, requiring considerable expertise and specialised equipment. Many of the organic acidaemias are able to be treated by either diet or replacement therapy. The long-term success of such therapy depends on careful clinical and laboratory monitoring and should be carried out at centres equipped to provide these special facilities. Similarly, the biochemical and supporting clinical experience required for the reliable diagnosis of inherited metabolic diseases suggests that such work should also be concentrated at specialised centres if the optimum detection and care of the affected individuals is to be achieved.

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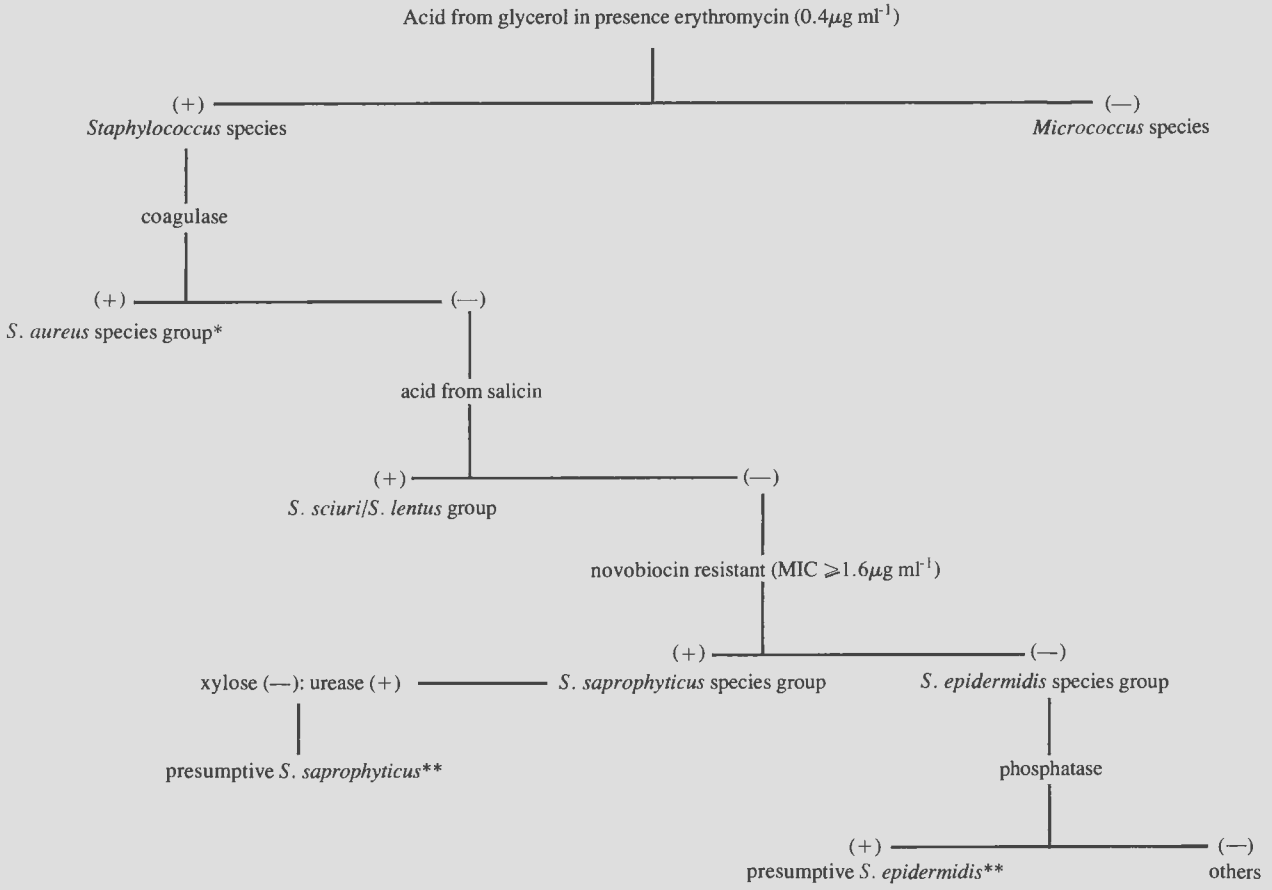
Table 3. Initial characterization of catalase positive, Gram positive cocci occurring singly, in pairs, or clumps



* Excluding *S. simulans*
** *See table 4*

Table 4. Separation of *S. epidermidis* and *S. saprophyticus* from other members of their respective species groups, and from other coagulase negative staphylococci

Test	Species									
	epidermidis	*hominis*	*haemolyticus*	*warneri*	*capitis*	*saprophyticus*	*cohnii*	*xylosus*	*simulans*	*auricularis***
Phosphatase	+*	(—)	(—)	(—)	(—)	—	(—)	(+)	(+)	—
Urease	+	+	—	+	—	+	—	+	+	—
Acid from:										
Mannitol	—	(—)	(+)	(+)	+	(+)	+	+	(+)	—
Xylose	—	—	—	—	—	—	—	+	—	—

* + and — indicates >80% strains positive and negative respectively; signs with brackets indicate >70%
** very slow growing; external ear canal inhabitant

promise as an epidemiological marker for clinical isolates of coagulase negative staphylococci[22]. Like slime formation, plasmid analysis, biotyping and phage typing are primarily research tools for strain identification although the latter two techniques would seem in practice of little use.

This information clearly indicates that it is no longer justifiable to simply lump all staphylococcal isolates which fail to clot plasma into a large coagulase negative group. How far therefore should we go in attempting to identify and speciate clinical isolates? Clearly computerized numerical taxonomic techniques, as carried out by Gunn and colleagues[10], are not presently feasible for the routine microbiology laboratory. Similarly it seems for most laboratories in New Zealand that the cost of commercially available API or DMS systems is prohibitive. In addition each of these commercial kits have shortfalls which may involve up to 20% of isolates[11]. Conventional identification schemes based on the methods of Kloos and Schleifer[1,7] are long and time consuming and possibly unwarranted on this basis. With these thoughts in mind, it would seem that the suggestion of Gunn's group that laboratories initially confine themselves to identifying new isolates at a *Staphylococcus* species group level rather than individual species per se is a satisfactory working hypothesis. An example of the tests which may achieve this aim are shown in tables 2 and 3.

Accumulated evidence would suggest that within the coagulase negative group, two species stand out as potential pathogens. These are *S. epidermidis*, which in one survey comprised 93% of clinically significant isolates from wounds and body fluids including urine[13], and the uropathogen *S. saprophyticus*. It would seem logical therefore to include additional tests which would enable the presumptive identification of these two species (table 3). Then again, a lesser

Table 5. Isolates of coagulase negative staphylococci to be further identified and tests to be undertaken

ISOLATES FROM:	— Normally sterile sites: e.g. blood, C.S.F., pleural and peritoneal fluids, internal prostheses
	— Catheter tips where >500 colony forming units recovered (i.e. "infected" tips)
	— Wounds where recovered in heavy growth if mixed, or moderate growth if pure.
	— Urines: (1) all isolates from bladder aspirates (2) isolates from mid stream and catheter specimens where $\geqslant 10^4$/ml*
TESTS:	— Acid from glycerol in presence of erythromycin (0.4μg ml^{-1})
	— Antibiotic sensitivity pattern: penicillin, methicillin, cephalothin, erythromycin, gentamicin, vancomycin
	— Slime production
	— Enzyme production: phosphatase, urease
	— Novobiocin sensitivity (MIC $\geqslant$1.6μg ml^{-1})
	— Acid form: mannitol, salicin, xylose

* See also text

alternative could be to speciate in this manner only those isolates which produced slime *in vitro!* Presumptive identification of other species would require the use of a series of additional tests (e.g. urease production, tween 80 hydrolysis, haemolytic action, nitrate reduction[10]) not all of which lend themselves well to automation. Considering the relative unimportance of these other species, this would seem impracticable for most laboratories. Although sugar fermentations have been widely employed in most staphylococcal taxonomic schemes, it seems from recent evidence[10] that this is too variable to be of pronounced use. Whether or not limiting identification of coagulase negative staphylococci to a species group level for isolates other than *S. epidermidis* and *S. saprophyticus* will turn out to be an unfortunately short sighted approach remains to be seen. Obviously introduction of this sort of identification system is going to increase the work load of the microbiology laboratory. Thus tests which can be adapted to multipoint inoculation techniques and/or overnight incubation hold most practical promise.

There is also the problem of which isolates should be subjected to further identification. In Dunedin, we have settled on the scheme shown in table 4 although isolates from urines still create some concern. Probably all isolates from mid stream and catheter-obtained specimens should be grouped, for levels of stahylococci below 10^4/ml can be asssociated with significant urinary tract pathology. However, if this were to be done in Dunedin at present, the number of isolates being identified to a species group level would be unacceptably high.

In conclusion, we see the scheme presented here as theoretically of use in the initial speciation of coagulase negative staphylococci potentially of clinical significance. Whether or not this will turn out to be true in practice is presently under investigation in Dunedin.

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CONTINUING EDUCATION

Revision Series in Biochemical Calculations
Section II: Molecular Weights

Trevor A. Walmsley
Dept. of Clinical Biochemistry, Christchurch Hospital, Christchurch.

DEFINITIONS

Atomic Weight

"Atomic weight is the relative weight of the atom on the basis of Carbon 12 as 12."

Some Useful Atomic Weights

Atom	Symbol	Atomic Weight	Atom	Symbol	Atomic Weight
Hydrogen	H	1	Phosphorus	P	31
Carbon	C	12	Sulphur	S	32
Nitrogen	N	14	Chlorine	Cl	35.5
Oxygen	O	16	Potassium	K	39
Sodium	Na	23	Calcium	Ca	40

For accurate work use IUPAC Atomic Weights 1979 (or later).

Avogadro's Number

"Avogadro's Number is the number of Carbon 12 atoms in 12 g of Carbon 12 and is approximately equal to 6.02×10^{23}".

1 mole of carbon weighs exactly 12 g (by definition) and contains approximately 6.02×10^{23} carbon atoms. Therefore 1 atom of carbon weighs approximately 2.0×10^{-23} g. Similarly 1 atom of potassium weighs approximately 6.5×10^{-23} g etc.

Molar Gas Volume

"The volume of 1 mole of an ideal gas is 22.4 litres at 0°C and 1 standard atmosphere pressure".

For example — how many oxygen molecules are present in 1 ml of oxygen at 0°C and 1 standard atmosphere pressure?

1 mole of oxygen (O_2) occupies 22.4 l

therefore 22.4 l of oxygen contains 1 mole of oxygen

22.4 l of oxygen contains 6.02×10^{23} oxygen molecules

therefore 1 ml of oxygen contains $\frac{6.02 \times 10^{23}}{22.4 \times 1000}$ oxygen molecules

$= 2.7 \times 10^{19}$ molecules

Molecular Weight

"Molecular weight is the sum of the atomic weights of all the atoms in the molecule.".

The unit of molecular weight is gram per mole of substance ($g.mol^{-1}$).

For example the molecular weight of water (H_2O) = 1+1+16 = 18. Therefore 18g of water (H_2O) contains 6.02×10^{23} molecules of water (H_2O) and a H_2O water molecule weighs approximately 3.0×10^{-23}g.

The mole

"The mole (symbol mol) is the amount of substance containing as many elementary entities as there are atoms in 12 g of Carbon 12. (i.e. Avogadro's Number). When the mole is used the elementary entities must be specified and may be atoms, molecules, ions or particles."

The number of moles present in a given mass is calculated by dividing the mass in grams by the molecular weight:-

$$\text{Moles} = \frac{\text{Mass (g)}}{\text{Molecular Weight } (g.mol^{-1})}$$

For example — to calculate the number of moles of NaCl in 30 g of Sodium Chloride (NaCl).

Molecular Weight NaCl = 23 + 35.5 = 58.5

$$\text{Moles of NaCl} = \frac{30g}{58.5\ g.mol^{-1}} = 0.513 \text{ mol NaCl} = 513 \text{ mmol NaCl}$$

Molarity

"The molarity of a solution is the number of moles present in 1 litre. A 1 molar solution is prepared by dissolving 1 mole of a substance in a solvent and then making the volume up to 1 litre."

In some disciplines of science mol*al*ity is used as a unit of concentration. A 1 mol*al* solution is prepared by dissolving 1 mole of a substance in 1 kilogram of solvent.

For example — to calculate the molarity of water (H_2O):-

The concentration of water = 1000 g/l

The molecular weight of H_2O = 1 + 1 + 16 = 18

$$\text{The molarity of water} = \frac{1000 \text{ moles/l}}{18} = 55.6 \text{ moles/l}$$

Therefore 1 litre of water contains 3.3×10^{25} water molecules. ($55.6 \times 6.02 \times 10^{23} = 3.3 \times 10^{25}$)

For example — to calculate the molarity of 0.9 g% sodium chloride solution (0.9 g/100 ml NaCl):-

Molecular weight of sodium chloride (NaCl) = 23 + 35.5 = 58.5

$$\text{Concentration NaCl} = 0.9 \text{ g/dl} = \frac{0.9}{58.5} \text{ moles/dl}$$

$$= \frac{0.9}{58.5} \times \frac{1}{100} \text{ moles/ml}$$

$$= \frac{0.9}{58.5} \times \frac{1000}{100} \text{ moles/l}$$

$$= 0.154 \text{ mol/l}$$

$$= 154 \text{ mmol/l}$$

For example — to calculate the weight of NaCl in 250ml of 150 mmol/l NaCl solution.

$$\text{No moles of NaCl in 1 ml of 150 mmol/l NaCl} = \frac{1}{1000} \times 150 \text{ mmol}$$

$$\text{No moles of NaCl in 250ml of 150 mmol/l NaCl} = \frac{250}{1000} \times 150 \text{ mmol}$$

$$\text{Weight of NaCl in 250 ml of 150 mmol/l NaCl} = \frac{250}{1000} \times 150 \times 58.5 \text{ mg}$$

$$= 2194 \text{ mg}$$

$$= 2.194 \text{ g}$$

Example:- How would you prepare 500 ml of an aqueous standard whose concentration is 150 mmol/l Na^+, 5 mmol/l K^+, 120 mmol/l Cl^- and 50 mmol/l CO_3^{--} from 1.5 mol/l NaCl, 100 mmol/l KCl, 1.0 mol/l NH_4Cl and 0.8 mol/l Na_2CO_3. To obtain the required concentrations in the final solution we require:-

NEW ZEALAND INSTITUTE OF MEDICAL LABORATORY TECHNOLOGY INC.

TREASURER'S REPORT

This year has traded with a substantial excess due to the success of the national Safety Seminar series conducted by Mr Mike Collins and the profit from the Napier conference.

The most significant increase in expenditure has been the Journal subsidy. The transfer of the Journal from Dunedin to Auckland has resulted in increased printing and administration costs and because of the price freeze we have been unable to increase the advertising charges. As we now undertake our own advertising management we are advised that we can expect an increased income from this source. We are also using bulk transport of Journal issues to hospitals for local redistribution which will produce a reduction in postage expenditure.

The Institute has an annual cash turnover in excess of $100,000.00 and has to be prepared to underwrite the annual conferences, including the eighth yearly South Pacific congress for which a reserve must be available to cope with any unexpected liability. It is not Council's wish to accumulate large reserves and indeed most annual budgets are programmed to operate at a loss. However, for the past few years invariably due to unexpected extra income conferences have produced surpluses of $2,000 to $3,000 annually. With the present established reserves Council now has the ability to proceed to organise with confidence regular Post Graduate Seminars and Workshops etc.

For this coming year Council has budgeted for a subsidiary of $12,000.00 for the Journal and $5,000 towards the running costs of Post Graduate Seminars and Workshops from an income of $44,500.00 and an expenditure of $47,000.00.

With the lifting of the price freeze there are many increases in routine expenditure to meet e.g. travel, accommodation, printing etc which I expect to amount to approximately 10% in this current financial year, which will result in a trading loss and thus I must in all responsibility recommend a subscription increase to commence with the next financial year. The new subscriptions as proposed, are expected to increase the total subscription income by approximately $5,000.00. For Staff Technologist Associate Members on the First Step of a Staff Technologist salary this new subscription levy will represent only 0.26% of their basic income.

W.J. Wilson
HONORARY TREASURER

NEW ZEALAND INSTITUTE OF MEDICAL LABORATORY TECHNOLOGY INC. STATEMENT OF FINANCIAL POSITION AS AT 31 MARCH, 1984

	1984	1983
ACCUMULATED FUNDS		
Balance as at 1 April 1983	42,085	42,937
Plus: Surplus (deficit) for the year	11,697	(852)
	53,782	42,085
Clinical Laboratory Special Fund	641	641
TOTAL INSTITUTE FUNDS AS AT 31 MARCH 1984	$54,423	$42,726
These funds were represented by the following items		
CURRENT ASSETS		
Cash at bank	23,774	10,894
Stock of examination stationery	50	50
Air New Zealand Bulkair Deposit Account	614	550
Stock (Ties/Badges/Cufflinks)	551	716
Conference advances	200	750
Sundry debtors	580	1,472
Subscriptions in arrears	1,925	351
TOTAL CURRENT ASSETS	27,694	14,783

LESS LIABILITIES		
Subscriptions in advance	283	222
Examination fees in advance	3,187	3,335
	3,470	3,557
NET CURRENT ASSETS	24,224	11,226
PLUS INVESTMENTS		
Mortgage investment	—	2,000
General Finance Ltd	15,000	15,000
BNZ Finance Ltd	13,000	8,000
BNZ Savings Bank Ltd	—	6,170
TOTAL INVESTMENTS	28,000	31,170
Fixed asset (at cost less depreciation) Typewriter	2,199	330
	$54,423	$42,726

The attached notes form part of this Statement

Treasurer — W.J. Wilson President — A.F. Harper

NEW ZEALAND INSTITUTE OF MEDICAL LABORATORY TECHNOLOGY INC. STATEMENT OF INCOME & EXPENDITURE FOR THE YEAR ENDED 31 MARCH, 1984

INCOME FOR THE YEAR WAS DERIVED FROM:	1984	1983
Subscriptions	38,247	32,356
Conference surplus (as per statement)	3,770	8,622
Interest received	5,449	4,561
Examination surplus	819	537
Miscellaneous Income	5,406	1,185
	53,691	47,261
FROM THIS INCOME THE FOLLOWING EXPENDITURE WAS MET:		
Accommodation etc	5,009	5,021
Fees — C.S.U., IAMLT and NCCLS	2,830	2,677
Journal cost (as per statement)	14,052	9,505
Honoraria, Gratuities & Prizes	2,684	1,666
Computer services	724	846
Printing stationery and typing	3,825	6,896
Postage & tolls	2,612	2,807
Travelling expenses	5,948	17,305
Post Grad. Education and Pacific Training	3,618	500
Sundry expenses	571	780
	41,873	48,003
Plus Depreciaton of typewriter	121	110
Total expenditure for year	41,994	48,113
Which leaves an excess of income over expenditure (1983 expenditure over income) for the year	$11,697	$ (852)

The attached notes form part of this statement

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5 mmol/l KCl this would give 5 mmol/l K^+ and 5 mmol/l Cl^-.
50 mmol/l Na_2CO_3 this would give 100 mmol/l Na^+ and 50 mmol/l CO_3^{--}.
50 mmol/l NaCl this would give 50 mmol/l Na^+ and 50 mmol/l Cl^-.
65 mmol/l NH_4Cl this would give 65 mmol/l NH_4^+ and 65 mmol/l Cl^-.

This would give a total of 150 mmol/l Na^+, 5 mmol/l K^+, 120 mmol/l Cl^- and 50 mmol/l CO_3^{--}.

Volume of 100 mmol/l KCl required to give 500 ml of 5 mmol/l KCl.

No moles KCl required = 2.5 mmol/500 ml

Since 100 mmol/l KCl contains 0.1 mmol/ml of KCl, 25 ml of 100 mmol/l KCl would contain 2.5 mmol KCl. Therefore we require 25 ml of 100 mmol/l KCl diluted to 500 ml to produce 500 ml of 5 mmol/l KCl

Alternatively in the general case where we have X ml of A mol/l solution diluted to Y ml of B mol/l, the number of moles of solute remains constant.

$$\text{No moles solute} = \frac{X \times A}{1000} = \frac{Y \times B}{1000}$$

therefore $X \times A = Y \times B$

In the above example we require X ml of 100 mmol/l KCl (A) diluted to 500 ml (Y) to give 5 mmol/l KCl (B)

X ml x 100 mmol/l = 500 ml x 5 mmol/l

$$X = \frac{500 \times 5}{100} = 25 \text{ ml}$$

Similarly we require X ml of 0.8 mol/l Na CO_3. However in order to use the simple equation we must express the concentrations in the same units.

X ml x 800 mmol/l = 500 ml x 50 mmol/l

$$X = \frac{500 \times 50}{800} = 31.25$$

Also we require X ml of 1500 mmol/l NaCl diluted to 500 ml to give 50 mmol/l NaCl.

X ml x 1500 mmol/l = 500 ml x 50 mmol/l

$$X = \frac{500 \times 150}{1500} = 16.7 \text{ ml}$$

Also we require X ml of 1000 mmol/l NH_4Cl diluted to 500 ml to give 65 mmol/l NH_4Cl.

X ml x 1000 mmol/l = 500 ml x 65 mmol/l

$$X = \frac{500 \times 65}{1000} = 32.5 \text{ ml}$$

Therefore we require 25 ml of 5 mmol/l KCl, 31.25 ml of 0.8 mol/l Na_2CO_3, 16.7 ml of 1.5 mol/l NaCl and 32.5 ml of 1.0 mol/l NH_4Cl diluted to 500 ml to give an aqueous standard whose concentration is 150 mmol/l Na^+, 5 mmol/l K^+, 120 mmol/l Cl^- and 50 mmol/l CO_3^{--}.

Section II — Problems (Answers on page 106).

1. Calculate the molecular weight of glucose ($C_6H_{12}O_6$).
2. Calculate the molecular weight of urea (CH_4N_2O).
3. Calculate the molecular weight of creatinine ($C_4H_7N_3O$).
4. Calculate the molecular weight of sodium hydroxide (NaOH).
5. Calculate the number of moles present in 180 g glucose
6. Calculate the number of moles present in 30 g urea.
7. Calculate the number of moles present in 113 mg creatinine.
8. Calculate the number of moles present in 10 g sodium hydroxide.
9. Calculate the molarity of 180 g/l glucose.
10. Calculate the molarity of 30 g/500 ml urea.
11. Calculate the molarity of 2 g/100 ml sodium hydroxide.
12. Calculate the molarity 90 mg/dl glucose.
13. Calculate the molarity of 11.3 mg/100 ml creatinine.
14. Calculate the molarity of 30 mg/dl urea.
15. What weight of glucose is present in 1 litre of 0.5 mol/l glucose?
16. What weight of sodium hydroxide is in 500 ml of 1.0 mol/l sodium hydroxide?
17. What weight of creatinine is present in 100 ml of 1.0 mmol/l creatinine?
18. What weight of urea is present in 250 ml of 10.0 mmol/l urea?
19. What weight of sodium chloride is in 100 ml of 1000 mmol/l sodium chloride?
20. A reagent you are preparing requires 164 g of anhydrous sodium acetate. You only have available sodium acetate trihydrate ($C_2H_3NaO_2.3(H_2O)$). What weight of sodium acetate trihydrate would you use in place of 164 g anhydrous sodium acetate?
21. Another reagent you are preparing requires 114 g di-potassium hydrogen phosphate trihydrate ($K_2HPO_4.3(H_2O)$). You only have available anhydrous di-potassium hydrogen phosphate. What weight of the anhydrous salt would you use in place of the trihydrate?
22. In a solution of 5.3 g/l sodium carbonate (Na_2CO_3) what is the molarity of (a) Na_2CO_3 (b) Na^+ (c) CO_3^{--}.
23. On mixing 100 ml of 0.1 mol/l calcium chloride ($CaCl_2$) with 100 ml of 0.1 mol/l sodium chloride (NaCl) what is the molarity of (a) Ca^{++} (b) Na^+ (c) Cl^-.
24. Given that the molecular weight of haemoglobin is 64 000 and that there are four iron atoms per molecule of haemoglobin. Calculate the molarity of hemoglobin iron in 15 g/dl haemoglobin. Express your answer in μmol/l.
25. Assuming that there is 1 bilirubin binding site per molecule of albumin (molecular weight 68 000) what is the maximum no of moles of bilirubin that 50 g/l albumin can bind? Express your answer in μmol/l.

Contaminant or Opportunist?

S.D.R. Lang and N.J. Beeching

Microbiology Laboratory, Middlemore Hospital, Auckland.

This paper discusses bacteria which are occasionally pathogenic but which may be inadvertantly labelled 'contaminants'. It reviews the circumstances in which these opportunists are likely to be significant and emphasizes a combined clinical and microbiological approach to ensure that they will be neither overlooked nor treated inappropriately. Details of microbiological identifications are not given but illustrative case histories are included.

Whether an organism causes disease depends as much on host defences as on its inherent virulence. Despite considerable advances in our understanding of both these factors, clinical microbiology remains almost exclusively empirical since its theoretical explanations lag far behind its ability to recognize relevant associations.

Table I lists some commonly encountered organisms of inherently low pathogenicity together with their natural habitats and the situations in which they cause disease. The organisms fall into two categories; commensals whose normal habitat is the skin or mucosa of man, e.g. *Staphylococcus epidermidis* and *Propionibacterium acnes*, and species which are common in the environment, e.g. *Pseudomonas* species and

Table 1
Examples of potentially pathogenic bacterial commensals and opportunists

ORGANISM	NATURAL HABITAT	INFECTIONS
Staphylococcus epidermidis	Skin and mucosa of man and animals ubiquitous	In relation to foreign bodies (stitches, prostheses, shunts and catheters), wound and joint infections, endocarditis, peritonitis, vaginitis, intravascular cannula-related sepsis and urinary tract infection
Other coagulase-negative staphylococci and *Micrococcus* species		Similar infections to *S. epidermidis* but much less commonly
viridans streptococci	Oropharynx and GI tract	Endocarditis (all species), less commonly otitis media, sinusitis, empyema, brain abscess, intra-abdominal abscesses (especially *S. milleri)* and septicaemia
Aerococcus species	Environmental	Poorly defined. Endocarditis, urinary infections
Corynebacterium species *Propionibacterium acnes*	Skin and mucosa of man and animals, soil and water	Infection associated with prosthetic devices, pleuro-pulmonary infections, endocarditis, abscesses and wounds
Bacillus species	Ubiquitous	Wound infections, septicaemia
Branhamella catarrhalis	Oropharynx	Sinusitis, otitis, bronchitis, pneumonia in the immunocompromised
Pseudomonas species *Acinetobacter* species *Aeromonas* species *Alcaligenes* species *Flavobacterium* species	Ubiquitous in moist environments. Some species colonise humans sufficiently often to be regarded as normal flora. Colonisation is increased by disease, hospitalisation and antibiotic therapy	Infections of multiple organ systems, especially in debilitated patients
Pasteurella multocida	Normal oral and respiratory flora of domestic animals	Wound infections following animal bites, broncho-pulmonary disease and systemic sepsis
Eikenella corrodens	Human oropharynx	Knuckle and human bite injuries
Vibrio species	Sea and brackish water	Wound infections

Bacillus species. The latter may first establish themselves as colonists, or be introduced immediately into a situation where they are pathogenic via contaminated solutions or prosthetic devices. Both commensals and environmental opportunists may contaminate laboratory media or specimens during their collection. They are usually recognized as pathogens when repeatedly isolated from normally sterile specimens or when found in pure culture in association with pus. They may be inappropriately dismissed if the microbiologist is not given full clinical particulars or is unaware of those situations in which they are likely to cause disease.

Predisposing host factors can also be regarded as falling into two categories. The immune system itself may be compromised, with generalised susceptibility to a variety of pathogens depending on whether the deficiency is one of phagocytic function (especially neutropenia), humoral immunity, complement, or cell-mediated immunity. The immunocompromised host is at increased risk from both opportunists and conventional pathogens[1]. Even the less common of the latter, e.g. *Listeria monocytogenes* or *Nocardia asteroides,* are unlikely to be discounted as irrelevant. More commonly, there is localized compromise due to breaching of mechanical barriers or insertion of cannulae, tubes or prostheses. These account for most opportunistic infections due to 'commensals' (Table II). In the hospitalised patient, predisposing factors are commonly multiple and the relative importance of each may be difficult to determine.

Low-grade pathogens which might be dismissed as commensals or contaminants

Infections caused by *S. epidermidis* exemplify the problems outlined above. In a recent review of blood cultures, *S. epidermidis* accounted for 18% (163/910) of isolates but was thought to be significant in only 10 cases[2]. Placement of venous lines (peripheral and central), intra-arterial lines and peritoneal dialysis catheters all carry a high risk of introducing *S. epidermidis* which accounts for about 20% of bacteraemias associated with intravenous catheters and approximately a third of cases of peritonitis in patients on continuous ambulatory peritoneal dialysis (CAPD)[3]. *S. epidermidis* commonly infects patients with cerebrospinal fluid (CSF) shunts, prosthetic joint implants, prosthetic heart valves and vascular grafts. It is of doubtful importance when found in routine catheter specimens of urine but may be a pathogen after prostatectomy. It is an unusual cause of wound infection in otherwise healthy patients. Although there are now at last 12 recognized species of coagulase-negative staphylococci, some laboratories report only *S. epidermidis* and, in the case of urinary isolates, *S. saprophyticus*. This has some justification as other species are much less commonly pathogenic[4,5]. Methicillin-resistant *S. epidermidis* may be both difficult to recognize and difficult to treat. Methicillin susceptibility should be determined using the same precautions as indicated for testing *S. aureus*[6,7]. Susceptibility to vancomycin, rifampicin and fusidic acid should always be determined. In the past, methicillin-resistance has been taken to imply resistance to all cephalosporins. This is not true *in vitro*[8], but the assumption is prudent until clincial studies have been performed.

Viridans streptococci as a group are the most common cause of subacute bacterial endocarditis and the association of *S. mutans* with dental caries is well known. In addition, *S. milleri* has emerged as a common cause of suppurative conditions such as subphrenic and hepatic abscesses, empyema and brain abscess[9,10].

CASE:

A 36 year old man presented with a four month history of weight loss of 30kg, malaise and vague left chest pain. He was febrile and had clinical signs of a large empyema from which three litres of pus were drained. Blood cultures were negative. Gram positive cocci present in the pus were subsequently identified as *S. MG-intermedius (S. milleri)* and parenteral penicillin was administered. Computerized tomography revealed a large abscess in the left lobe of the liver with extension into the subphrenic space. At laparotomy an inflamed Meckel's diverticulum was found to be the source of infection. Post-operative recovery was uneventful.

Other viridans streptococci are seldom pathogenic but are capable of causing serious disease in immunocompromised patients, as illustrated by a recent report of septicaemic episodes in leukaemic patients, from whom *S. mitis* was the species most frequently isolated[11].

'Diphtheroids', together with *S. epidermidis,* are the most common contaminants of normally sterile body fluids collected by puncturing the skin. The frequency with which they are encountered has led, in the past, to important but relatively uncommon pathogens being overlooked. *Listeria monocytogenes, Erysipelothrix rhusiopathiae, Nocardia* species, atypical Mycobacteria and *Corynebacterium diphtheriae* itself are Gram positive non-sporing bacilli morphologically similar to, or indistinguishable from 'diphtheroids'. *Cardiobacterium hominis* and *Gardnerella vaginalis* are Gram negative but characteristically retain sufficient methyl violet to cause confusion. The bulbous ends of *C. hominis* and the usual association of *G. vaginalis* with infection arising from the female genital tract are useful clues. Awareness of these organisms is now such that they are unlikely to be discounted inappropriately or identified incorrectly.

CASE:

A 24 year old woman presented with fever, rigors, headache and neck stiffness of 5 days duration. A soft systolic bruit was noted together with

Table II
Procedures and prostheses predisposing to infection with low grade pathogens (adapted from Sen et al (1))

PROCEDURE AND/OR DEVICE	OPPORTUNISTIC PATHOGENS	SPECIMENS CULTURED
Intravascular catheter	*Staphylococcus epidermidis* 'diphtheroids' *Bacillus* species Gram negative bacilli *Candida* species	Blood Catheter tip Insertion site swab
Contaminated infusate	*Pseudomonas* species *Citrobacter* species *Enterobacter* species *Flavobacterium* species	Blood Catheter tip Infusate
Haemodialysis shunt	*Staphylococcus epidermidis* 'diphtheroids' Gram negative bacilli	Site swab Blood
Peritoneal dialysis catheter	*Staphylococcus epidermidis* viridans streptococci Gram negative bacilli anaerobes	Dialysate
Cerebro-spinal fluid shunt	*Staphylococcus epidermidis* 'diphtheroids' *Propionibacterium acnes* *Bacillus* species Gram negative bacilli	Cerebrospinal fluid Blood Peritoneal fluid
Prosthetic heart valves and cardiac pacemakers	*Staphylococcus epidermidis* 'diphtheroids' viridans streptococci Gram negative bacilli *Candida* species and other fungi	Blood Surgical specimens
Vascular grafts and aortic aneurysms	As for prosthetic valves plus *Salmonella enteritidis*	Blood Surgical specimens
Joint prostheses	*Staphylococcus epidermidis* 'diphtheroids' viridans streptococci Gram negative bacilli	Joint aspirate Tissue Blood
Contact and intraocular lenses	*Pseudomonas* species other Gram negative bacilli	Eye swabs and aspirates Disinfectants

petechiae and microscopic haematuria. Her spleen was not palpable on presentation but later enlarged. Three sets of blood cultures grew a Gram positive bacillus identified as toxigenic *C. diphtheriae* var. *gravis*. Nasopharyngeal swabs were negative. C.S.F. showed a modest pleocytosis consistent with emboli and grew *S. epidermidis* believed to be a contaminant. Large mitral valve vegetations were subsequently demonstrated by echocardiography. Her course was complicated by repeated major emboli and fever which persisted into the fourth week but she eventually recovered on intravenous penicillin. *C. diphtheriae* was recovered from nasopharyngeal swabs from her son and from a friend who had recently travelled out of New Zealand. All had received diphtheria immunization and had no symptoms attributable to the toxin.

'Diphtheroids' designated 'Group JK'[12] are notable both for their propensity to cause serious disease, usually in immunosuppressed patients and especially in association with intravascular lines, and for their remarkable resistance to most antibiotics. 'Group JK' organisms are uniformly susceptible to only vancomycin, but this important clue to their identification will be missed if susceptibility tests are not performed. However, these organisms are also commonly recovered as colonists or contaminants. In a recent study of 6,859 clinical specimens, 72 isolates of 'Group JK' were recovered from 65 patients, only two of whom were regarded as having infection due to these organisms[13]. Other antibiotic-resistant 'diphtheroids' have also been recovered from patients immunocompromised by malignancy[14].

Most *Corynebacterium* species are relatively susceptible to antibiotics. These and related genera including *Rothia, Kurthia, Rhodococcus, Lactobacillus* and the anaerobe *Propionibacterium acnes* are occasionally pathogenic. For example, *C. equi* (also known as *Rhodococcus equi),* was first described as a cause of pneumonia in foals but also infects cattle, swine and sheep producing lymphadenitis, mediastinitis and pyometra. Its natural habitat is soil and when grown in the diagnostic microbiology laboratory it is usually regarded as a contaminant. However, it is also a cause of pulmonary infection in immunosuppressed patients[15].

CASE:

A 52 year old man with diffuse histiocytic lymphoma was admitted with pleuritic pain, fever and dyspnoea. *C. equi* was isolated in pure culture from his empyema. It formed characteristic mucoid, salmon-pink colonies after prolonged incubation. Although this infection was eradicated by drainage and prolonged antibiotic therapy, the patient subsequently died of his underlying disease[16].

Bacillus species are ubiquitous in nature and are among the most common of plate contaminants. With the exceptions of *B. cereus*, which is a cause of food-borne gastroenteritis, and of *B. anthracis* which is unlikely to be encountered in New Zealand, *Bacillus* species are much more likely to be contaminants than the cause of disease. However, *B. subtilis, B. cereus* and several other species have occasionally been incriminated in wound infections and serious sepsis including meningitis, septicaemia, endocarditis and pneumonia[17].

Branhamella catarrhalis was, until recently, regarded as a non-pathogenic commensal of the upper respiratory tract but is now recognized to be a significant cause of sinusitis, otitis media, acute exacerbations of bronchitis and of pneumonia in the immuno-compromised. It is an occasional cause of empyema, endocarditis and meningitis. It is invariably present in expectorated sputum and transtracheal aspiration has been advocated to confirm its presence in the lower respiratory tract[18]. In our experience a Gram stain of expectorated sputum will usually suffice as the organism is easily seen within polymorphonuclear leukocytes. Its recognition is important as most strains produce beta-lactamase.

Gram negative bacilli present a different problem from Gram positive opportunists. Most laboratories take great pains to report the isolation of Gram negative bacilli and speciate them even if this means referral to a reference laboratory. While this is certainly justified when organisms are isolated in pure culture from a normally sterile site, it is over-zealous when they are recovered from mixed wound infections, from around endotracheal tubes or from the inanimate environment. There are several possible reasons for this cautious approach: the diversity of species of Gram negative bacilli; an established tradition of speciating especially the *Enterobacteriaciae* and the availability of convenient schemata for this purpose; recognition that these organisms are not commensals of the skin and seldom colonise the oropharynx of healthy persons outside hospital; and the frequent morphological and biochemical similarity of opportunists to well-recognized pathogens.

Many of these organisms do colonise ill, hospitalised patients but in the majority of cases do not go on to cause disease. Surveillance cultures are of little value except when dealing with neonates and neutropenic patients. There is a tendency to over-interpret the importance of Gram negative bacilli found in mixed culture from colonised areas, and to over-prescribe antibiotics in an effort (usually futile) to sterilize the site. These comments are not intended to discourage the speciation of Gram negative bacilli, but rather to indicate that their importance is unlikely to be overlooked unless they are either slow growing, fastidious or both. An example of one such organism is *Eikenella corrodens,* which is commonly incriminated in wound infections following human bites[19].

Procedures and prostheses which predispose to opportunistic infections

The insertion of a peripheral intravenous line is so common-place that it is frequently carried out in a cavalier fashion by the most junior medical staff. Long-term central venous lines for cardiovascular monitoring are being used more and more in the management of seriously ill patients. Even a low rate of catheter-related sepsis translates as a significant economic burden and threatens the lives of those in whom it occurs. Up to one third of all hospital acquired bacteremias derive from vascular catheters[20]. Most catheter-related sepsis results from ascending infection from the cutaneous entry point but unfortunately correlates poorly with local signs of inflammation. Colonisation of the catheter tip secondary to bacteremia from another source is relatively uncommon.

Catheter tip culture is a semiquantitative procedure since contamination is almost inevitable when the catheter is withdrawn. Maki et al[21] have shown that rolling the distal few centimetres of catheter over solid media is an easy and reliable method. Catheters associated

with septicaemia yield at least 15 colonies and more commonly confluent growth, whereas contamination should result in fewer than 15 colonies. Since bacterial colonisation occurs in association with platelet-fibrin thrombi on the outside surface, it is not necessary to flush the lumen. Anaerobes are seldom involved in catheter sepsis and routine anaerobic culture is not warranted. *Candida albicans* has been implicated particularly when lipids are given during parenteral hyperalimentation. It has been suggested that comparison of quantitative cultures taken via the catheter in situ and a separate venepuncture may establish whether the catheter is colonised without it being withdrawn[22]. In our hands this has not been reliable as samples drawn through the catheter are frequently contaminated, possibly by organisms on the hub[23].

Contamination of the infusate itself is uncommon. Intrinsic contamination due to faulty manufacture or bulk dispensing of intravenous fluids may present as an epidemic of infusion-related sepsis due to opportunistic hydrophilic pathogens capable of attaining large numbers at room temperature. In the case of blood or blood products which have been stored in a refrigerator, psychrophilic bacteria capable of proliferating at 4°C should be suspected.

Haemodialysis and peritoneal dialysis have both been associated with infections arising from shunt sites, dialysis machines or fluid and additives. Peritonitis develops in about one per cent of patients undergoing peritoneal dialysis and rates approximate to one episode per patient per year in those on CAPD. *S. epidermidis* and non fermentative Gram negative bacilli are commonly incriminated in addition to obvious pathogens such as *S. aureus* and *Enterobacteriaceae*. Peritonitis gives rise to abdominal pain in about 80% of cases and the dialysate fluid is almost always cloudy with white blood counts exceeding 100×10^6/litre. Some laboratories do not inoculate anaerobic plates unless the aerobic culture is negative, the patient is not responding to treatment, or bowel perforation is suspected. However, a broth medium capable of supporting anaerobes is essential as, although uncommon, anaerobic peritonitis has a serious prognosis[24].

CSF shunts are complicated by infection in up to one third of patients. Skin flora is usually involved, especially *S. epidermidis* and *S. aureus* and less commonly 'diphtheroids' or *P. acnes*. Gram negative infection has been associated with shunts inserted in patients with meningomyelocoele or ventriculitis and in those with ventriculo-ureteral shunts. Ventriculo-peritoneal and ventriculo-atrial shunts are more often used. Blood cultures are more commonly positive in the latter. The mortality of shunt infection may be as high as 40% and shunt removal in addition to appropriate antibiotics is generally indicated[1,25].

CASE:

A young man with a ventriculo-peritoneal shunt inserted to control hydrocephalus was admitted with headache, minimal neck stiffness and a low grade fever. Lumbar puncture yielded CSF with 7×10^6 lymphocytes per litre, slightly raised protein, and borderline low glucose. No clinical particulars were provided, no organisms were seen on direct examination and when *P. acnes* was recovered on culture it was discounted as a contaminant. The correct diagnosis of shunt infection due to *P. acnes* was not made until the same organism had been recovered from two further CSF specimens drawn several weeks apart. His symptoms resolved promptly with antibiotic therapy and shunt replacement.

Endocarditis and endarteritis produce continuous bacteremia and intravascular infection is likely to present to the laboratory technologist as multiple positive blood cultures. Prosthetic valves contaminated prior to or during insertion generally cause symptoms with the first eight weeks. 'Early' endocarditis is most commonly due to *S. epidermidis* which seldom involves native valves[6]. 'Late' infection is more commonly due to viridans streptococci, but there is considerable overlap and it is now always clear whether infection indicates failure of prophylaxis during surgery, or arises from subsequent transient bacteremia[26,27]. Virtually any organism has the potential to infect prosthetic valves and mixed infections also occur. Apparent 'contaminants' should be regarded with the utmost suspicion in this clinical setting. Since clinical particulars are sometimes omitted from request forms, it is prudent to stock representative isolates recovered from more than one blood culture regardless of their apparent insignificance at the time. There is a particular association between *Salmonella enteritidis* and infection of diseased or grafted aortas. Persistent bacteremia due to this organism is unusual and should alert the physician to the possibility of aortic involvement[28].

Prosthetic joints, particularly of the hip and knee, are relatively massive foreign bodies whose insertion is inevitably associated with considerable tissue damage. It is a tribute to modern surgical technique that less than one per cent are now complicated by infection. Although *S. aureus* is the most common pathogen, *S. epidermidis* is implicated in 5-25% and streptococci of various groups in 3-40%. Infections due to Gram negative bacteria and polymicrobial infections are less common. Intraoperative cultures from non-infected hips are contaminated in up to 30% of cases. Therefore multiple specimens should be taken in cases of suspected sepsis that come to reoperation. Recovery of the same organism from all cultures is a useful means of distinguishing indolent infection from contamination. In many cases it is impossible to eradicate infection without removing the prosthesis, replacement of which is a formidable procedure[29].

Contact and intraocular lenses are prostheses of very different sorts: one is commonplace, inserted and removed by the user at will and fortunately bathed in the antibacterial solution known as tears; the other involves surgical insertion of a permanent foreign body into an area relatively isolated from host defences. Both are associated with infection due to opportunistic bacteria, especially Gram negative bacilli. In the case of contact lenses, the source is commonly the antiseptic solution used to store the lenses overnight. This may become heavily contaminated by *Pseudomonas* species and faecal flora. Intriguingly, the same organisms involve intraocular lenses to a greater extent than does *S. epidermidis* which can be regarded as normal, if transient, flora of the conjunctiva[1].

In conclusion, it is frequently difficult to distinguish between contaminants, commensals or colonists on the one hand and opportunistic pathogens on the other. Cost-effective microbiology does not allow for isolation, identification, susceptibility testing and reporting of every isolate, which in itself can mislead clinicians and lead to inappropriate therapy. Intelligent short-cuts must be taken to ensure efficient use of laboratory resources. These are only workable if laboratory personnel are aware of situations in which isolates may have special significance, as described in this article. Clinicians must recognize that when they submit a specimen, they are consulting expert colleagues, and should provide relevant clinical details. Under these circumstances there will be adequate exchange of information, mutual education and improved patient care. The versatility of our microbial adversaries challenges us to pool our resources as effectively as possible.

Self-assessment cases (answers on page 106).

Case 1

An 18 year old youth injured the second knuckle of his right hand during a fist-fight in the pub. Three days later he had local throbbing pain, marked cellulitis and enlargement of epitrochlear lymph nodes. Culture at 48 hours yielded 'mixed anaerobes and viridans streptococci'. He began oral antibiotic therapy but showed no improvement after 5 days. The lesion was re-swabbed, and after 48 hours minute pitting colonies were noted on both aerobic/CO_2 and anaerobic blood plates.

QUESTIONS

(a) What is the principal differential diagnosis when minute pitting colonies are seen on anaerobic blood plates?
(b) What characteristic odour may suggest the presence of this organism even when over-grown by other flora?
(c) Which two commonly prescribed groups of antibiotics are uniformly ineffective against this organism?
(d) Is the statement 'This organism is a low grade pathogen found in association with infections only in mixed culture' true or false?

Case 2

A 34 year old woman undergoing pre-transplant haemodialysis developed pain and tenderness at the site of her dialysis shunt. A site swab showed numerous pus cells and Gram positive cocci in clusters. Intravenous cloxacillin was begun. A coagulase-negative staphylococcus was recovered in heavy growth. Disc susceptibility testing after overnight incubation at 37°C showed susceptibility to methicillin and cephalothin but resistance to penicillin, clindamycin, erythromycin and gentamicin. After 48 hours of therapy there was no clinical improvement.

QUESTIONS

(a) 'The isolate is probably a contaminant commensal and further culture should be obtained to recover the pathogen'. True or false?
(b) 'The isolate will have been eradicated but superinfection by another organism is likely to have become established within 48 hours'. True or false?

(c) 'The coagulase-negative staphylococcus has mutated to resistance'. True or false?
(d) 'When a foreign body is *in situ* it is futile to attempt to eradicate infection without first removing the foreign material'. True or false?
(e) 'The method of susceptibility testing was invalid for *S. epidermidis*'. True or false?
(f) What other antibiotics should be considered?

Case 3

Over the course of 3 weeks *Pseudomonas cepacia* was recovered from blood cultures from 5 febrile patients in an intensive care unit. In two patients fever resolved without antibiotic therapy. All eventually recovered.

QUESTIONS

(a) The single most likely source of the organism is:
  (i) Laboratory contamination
  (ii) Respiratory equipment
  (iii) Hands of intensive care personnel dressing intravenous cannula sites
  (iv) Catheter-related urinary infection
  (v) Intravenous fluids
(b) Appropriate epidemiological measures include which of the following?
  (i) Immediate closure of the unit
  (ii) Hand, groin and axillary swabs of all staff
  (iii) Swabbing of sinks in the unit
  (iv) Culture of intravenous fluid bottles of the same batch used in infected patients
  (v) Review and culture of intravenous additives provided by the hospital pharmacy

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NEWS FROM THE HILL

Quality Control System for Medical Laboratories

Government is to give an annual grant of $120,000 for the next five years, to the Testing Laboratory Registration Council of New Zealand, the Minister of Health, Mr Aussie Malcolm announced recently. The grant, which will total $600,000 will be used by the Council to set up an external quality control system for medical laboratories in New Zealand.

"Quality control of laboratories is an essential part of a good primary health care system," said Mr Malcolm. "It is important to ensure that when laboratories carry out tests on patient specimens referred from General Practitioners or from other sources, that errors in testing these samples are reduced to an absolute minimum," the Minister added.

Mr Malcolm explained that at the moment, overseas testing laboratories carried out quality control of New Zealand laboratories. This system was not entirely satisfactory as although they may detect mistakes in testing procedures at New Zealand laboratories, because of their remoteness, little assistance was available on how to put things right.

"With the aid of this grant, The New Zealand Testing Laboratory Council will not only do the quality control but will be able to help laboratories on how to correct their procedures if too many errors are found in their tests," said Mr Malcolm.

Mr Malcolm added that overseas quality control of New Zealand laboratories was costing this country money. "The fact that quality control will now be done in New Zealand and done more effectively, illustrates yet again that in times of economic restraint, the health services need not suffer, and can be improved, if we learn to make better use of available resources," he said.

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Membership Sub-Committee Report May 1984

Membership

Since our March meeting there have been the following changes:

	May 84	March 84	Feb 83
Membership as at 17 May 1984	1326	1275	1470
LESS Resignations 25, G.N.A. 4	29	18	138
	1297	1257	1332
PLUS Membership Applications	45	69	51
TOTAL MEMBERSHIP	1342	1326	1383

Applications for Membership

Miss S. Adam	Auckland
Miss P. L. Bishop	Hamilton
Ms R. J.Carter	Christchurch
Mr K. F. Cooper	Auckland
Mr N. Cox	Northland
Miss D. H. Davies	Christchurch
Mr J. P. Fetherston	Whakatane
Mr B. G. Lockwood	Dunedin
Miss T. Mackay	Auckland
Miss G. J. McKenzie	Whangarei
Mr L. K. McKnight	Wellington
Miss N. T. McWhinnie	Nelson
Miss B. A. Moroney	Hastings
Miss A. M. Patton	Dunedin
Mr S. Paul	Wanganui
Miss R. L. Saunders	Whangarei
Mr M. Smith	Whangarei
Mr J. Spaans	Auckland
Mrs K. A. P. Stenhouse	Lower Hutt
Miss L. A. Terveen	Hamilton
Miss L. J. Thorby	Hamilton
Miss L. M. Truscott	Hamilton
Mr S. R. Walker	Dunedin
Miss L. J. Webster	Dunedin
Miss C. L. White	Kaitaia
Mr P. N. Wildbore	Palmerston North
Miss M. I. Bray	Palmerston North
Miss B. C. Meeuwesen	Dunedin
Miss T. M. Hurunui	Christchurch
Ms M. C. Hay	Wellington
Mr M. J. Denigan	Northland
Mr J. Gerards	Rotorua
Ms P. D. Bellaney	Auckland

Applications for Associateship

Mrs M. I. Carr	Wellington
Mr K. O. Chan	Hong Kong
Mr R. G. Chapman	Christchurch
Mr R. L. Hewett	Auckland
Mr B. Holliss	Auckland
Miss P. C. Jenkins	Lower Hutt
Mrs V.A. Malcolm-Smith	Auckland
Mr P. C. May	Christchurch
Mrs J. C. Nimmo	Christchurch
Ms P. Whelan	Christchurch
Mrs H. J. Young	Palmerston North
Mrs M. L. Hilbourne	Auckland
Miss D. E. Ewens	Auckland
Mrs P. C. Flaws	Christchurch
Mr M. J. Geurts	Hamilton
Miss F. J. Glassey	Ashburton
Mrs R. H. Haworth	Palmerston North
Mrs C. M. Hill	Christchurch
Miss M. H. Huymans	Auckland
Mrs W. M. Jackson	Hamilton
Miss L. J. Kape	Hamilton
Mrs E. Lyons	Auckland
Miss D. M. MacDonald	New Plymouth
Miss P.W. McComb	Otahuhu
Miss J. R. McDowell	Invercargill
Mrs N. A. Mason	Invercargill
Miss K. M. P. Mayes	Nelson
Mr A. C. Mee	Christchurch
Miss D. Meek	Dunedin
Mr C. J. Miller	Wellington
Miss K. A. Milne	Wellington
Miss W. M. Mitchener	Auckland
Mrs H. M. Muir	Palmerston North
Mrs J. M. O'Connor	Invercargill

Mrs H. B. Orton	Auckland
Miss J. A. Peters	Dunedin
Miss L. L. Robb	Otahuhu
Mr D. J. Scarrow	Hamilton
Miss S. J. Sexton	Dunedin
Miss S. J. Smith	Christchurch
Mr M. C. Thompson	Palmerston North
Miss C. J. Tollemache	Auckland
Miss C. E. Tulloch	Auckland
Mr P. J. Wakem	Wellington
Miss D. M. Wallace	Lower Hutt
Miss M. F. Wallace	Hamilton
Miss K. E. Wedd	Hamilton
Mr P. D. Edwards	Dunedin
Miss J. B. Alexander	Napier
Miss B. M. Anderson	Hamilton
Mr I. H. Barlow	Auckland
Mr G. M. T. Brandsen	Invercargill
Mrs V. Bryant	Tokoroa
Mrs R. J. Caldwell	Auckland
Ms R. A. Campbell	Wellington
Mr M. W. Crowther	Auckland
Mrs F. W. Curley	Lower Hutt
Mrs J. R. Deroles-Main	Wellington
Mrs J. L. Diprose	Auckland
Miss C. A. Dyson	Auckland
Miss D. K. Ellis	Invercargill

Resignations

Miss A. McNicholas
Mr B. Thackeray
Ms H. Henderson
Mrs M. C. Dominick
Miss K. D. Henderson
Ms J. Blythe
Ms F. Taylor
Miss F. J. McKinnon
Miss P. J. Blake
Mrs D. M. Coburn
Miss L. A. Rudkin
Mr B. Sweet
Miss C. A. Curtis
Miss H. Mynen
Miss S. P. Shine
Mr R. A. M. Anderson
Mr P. G. Hocquard
Mrs A. J. Wood
Mrs J. M. Compton
Miss N. Dent
Ms R. Campbell
Ms D. Meek
Mr R. T. Sheldon
Miss L. Anderson

Gone — No Address

Mrs Y. S. Halstead	Australia
Mr A. D. Thomas	Auckland
Mrs J. R. Blythe	Palmerston North
Mrs C. B. Pickett	Dunedin

BRANCH NEWS

DUNEDIN BRANCH

New Executive Members for 1984-85:

Chairman	— Mr R. Fyfe
Secretary	— Mr J. Robinson
Treasurer	— Miss J. Duncan
Committee	— Mrs G. Boyd
	Miss B. Meevwesen
	Mr J. Cross

Chairman's Report for 1983-84

The year commenced with the Awards Dinner and Dance held in the Town House on the 27th May. The guest speaker was Mike Collins who had just completed a successful Laboratory Safety course in Dunedin. Mike's humorous talk succeeded in catching and holding everyone's attention. The night ended with the band providing good dance music.

This was followed by a Wine and Pizza evening held on the 6th July. On this occasion Jeff Dwyer, the Chief Flying Instructor at Otago Aero Club, gave a talk on aviation in the community.

On the 11th August a Ten-Pin Bowling evening was held. Attendance was high and all enjoyed themselves.

The Otago Polytechnic trainee restaurant was the venue for the next functions held on the 20th October and the 3rd November. All those involved dined well in the pleasant surroundings.

Unfortunately the yearly Guy Fawkes celebration of fireworks and Bar-B-Que at Woodhaugh Gardens was eventually cancelled due to the weather.

The final function of the year, held on December 21st, was the laboratories Christmas Party. This reflected the overall success of the year's function organised by the Committee and the amount of work that some members were prepared to offer to ensure their success.

It must also be noted the extremely large amount of work which has already gone into the organisation of the 40th Annual Scientific Meeting of the Institute which is to be held in Dunedin during the 15-17th of August.

CHRISTCHURCH BRANCH

At the Annual General Meeting of the Christchurch Branch of the NZIMLT, the following were elected as members of the committee:

Chairman	— K. Ahern
Secretary	— M. Murnane
Treasurer	— A. McDury
Committee	— B. Hobson
	J. Aitken
	H. Potter

BOOK REVIEWS

Clinical Laboratory Science in Europe, the Roles of Industry, the Professions and Health Authorities. (ECCLS)

Edited by Stanley S. Brown, Derek H. Calam and E. Gloag.
Published by European Committee for Clinical Laboratory Standards
Price £9.50

This volume covers some 240 pages and 70 papers. The contributions included provide a valuable resource about European Laboratory Science for this decade and a view of likely future developments. I commend this publication as it provides insight for those wanting to understand the complexity of the system in which we work and play a part.

The European Committee for Clinical Laboratory Standards, was inaugurated in 1977. The aim of this organisation is for single standards to be accepted world wide. Before the first standards appeared, the opportunity was taken to hold a series of three seminars involving all three constituent groups i.e. health agencies, professions and industry.

The first seminar on industry and the clinical laboratory was held in Canterbury, England, on 31st March, 1981. The second seminar met in Lyon, France in November 1981. A book review of this meeting is recorded in the N.Z.J. Med. Lab. Technol. April, 1982, page 49. The third seminar on health agencies and clinical laboratory science was held in Bilthoven, Netherlands, in June 1982. The scene is set by a review of 'needs' from different viewpoints, such as the clinician and the industry. Four papers then review industry's approach to satisfying the clinical laboratory needs. This includes marketing, service back-up for reagents and equipment and the production process. The next section covers "industry and its environment". Papers presented on behalf of industry, the profession and health agencies express viewpoints regarding one another. Reagents, instrumentation and laboratory financing receive special attention. The trading environment attracts attention and in turn develops into a more indepth discussion of the introduction of new products. The final seminar is particularly concerned with "supra national bodies", organisational structures existing in various countries, quality assurance, funding and last, but by no means least, co-operation between various sectors. The concluding remarks expressed by Irene Batty sum up the ECCLS. "The concept that communication, consultation, collaboration and co-operation with all relevant like-minded organisations is a most useful and rewarding activity and should help us to the goal of a single standard, implemented by all partners in our area of health care — worldwide, is strongly endorsed by the membership of ECCLS.

John Powell.

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Confidence

- **Proven Accuracy:** linear correlation with hemagglutination inhibition results
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Circle 55 on Readers Reply Card

Mrs Savitri Raju (left) and Miss Stella Driu at work in the Microbiology section at Lautoka Hospital, Fiji.

Laboratory at Northern District Hospital. An outpatient having a blood test at Northern District Hospital, Santo, Vanuatu.

Fiji was next on the list and I visited both the Commonwealth Memorial (CWM) Hospital in Suva, and Lautoka Hospital in Lautoka (near Nadi). Both of these hospital laboratories are larger than that in Vaiola, with staff numbering approximately 35 and 17 respectively. All technicians in both labs have the Certificate of Medical Technology — a three year training programme organised through the Fiji School of Medicine (FSM). These laboratories also have a few expatriate technicians — either Peace Corp from America or Volunteer Service Overseas (VSO) from Britain.

Both laboratories I visited in Fiji said they would greatly appreciate a New Zealand technologist giving about 4 weeks of specialised lectures in Fiji, where many of their technical officers would be able to attend. I felt that this would be a good way New Zealand could be of assistance to Fiji.

Vanuatu recently gained its independence, it has 3 official languages — French, English and Bislama (Pidjin). Consequently, some of the laboratory staff do not speak English, so they are limited in training programmes they are able to attend. Some English speaking technicians have been on several overseas programmes, including the technicians course at Fiji, WHO courses and P.P.T.C., but those who do not speak English have only bench training (or nursing training). Whilst in Vanuatu, I spent several days at the Vila Base Hospital in Vila. They have a very good Blood Donor Service, organised by the Charge Technologist, Mr Kalorib Daniel, which includes a one hour radio programme each week and weekly Blood Mobile drives. This laboratory has about 8-10 staff, including a New Zealand technologist with V.S.A.

I flew to a northern island in Vanuatu for one day to visit a laboratory that is sending a technician to the next P.P.T.C. Blood bank course. This laboratory is the size of a small lounge, has three technicians who not only do all the laboratory work, but bleed the continual stream of outpatients, as well as 500-600 donors annually. The chief medical officer took the laboratory staff and myself out to lunch at the local Chinese restaurant, and it was good to be able to talk to someone from the medical staff to find out their requirements in relation to the laboratory work.

The Laboratory staff at Vila Base Hospital, Vanuatu.
Ruth Hornblow, a New Zealand V.S.A. worker and Andrea Hall, co-ordinator/tutor of the P.P.T.C. are in the front row.

The Solomons. Fortunately the rain kept the temperature low enough to be bearable for me but I still found that the way to keep comfortable was to go in a taxi or **walk** very slowly! Central Hospital in Honiara, like many laboratories I saw, is modern and nicely set out, with a pleasant view from the windows. They have a staff of 18, with seven overseas on two or three year training programmes. In the Solomons, as in Vanuatu, blood film examination for malarial parasites is an important part of haematology work, with some special laboratories being set aside just for malarial examinations.

Whilst in the Solomons, I took a day flight over Iron Bottom Sound to the island of Malaita, where at Kilu'ufi Hospital are 3 laboratory technicians, two having trained at the P.P.T.C. The hospital is small, but there seems to be an excellent relationship between laboratory and medical staff. I spent some time talking to the medical staff and they are happy not only with the number of tests being done (which is not a lot) but also the standard of work being produced. Any problems or queries the doctors may have with the work is discussed with the lab staff. I feel it is a shame this kind of relationship is lost in large hospitals.

As several bridges had been washed out with the recent rains, there were very few outpatients and consequently not much work to do, so we were able to sit and talk most of the day and I met the families of the two ex P.P.T.C. students, Wilfred Kiriau and Anthony Rickimae.

Papua New Guinea was the last country I visited. Port Moresby General Hospital (P.M.G.H.) lab is about the same size as C.W.M. in Suva. All staff here have had three or five years training. I was also able to visit the School of Allied Health Sciences on the P.M.G.H. campus. The school runs several programmes including the three year Technicians Certificate and following on from that, a two year full time Technologists Diploma. This school is staffed by locals and expatriates.

After a 7.30 a.m. departure from Port Moresby on 10th April, I arrived back in New Zealand, out of warm tropical weather into a cold 17 degrees!

My overall impression:

Most of the laboratories are fairly modern, spacious with pleasant working conditions, with tests and equipment showing great variation from laboratory to laboratory.

Each laboratory has its own requirements and so, I feel, effective aid can be provided in many ways, for example:

1. Small projects such as provision of labels for Blood Bags, redirecting New Zealand labs' unused equipment and books (where suitable) to labs requiring them.
2. Involvement in Quality Control Programmes.
3. A New Zealand technologist travelling to the Pacific to give 4-6 weeks specialised teaching.
4. Sponsoring technicians for training in New Zealand — either at the P.P.T.C. or in some cases in hospital laboratories for three months.
5. Encouragement of communication between Pacific Island

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laboratories and each other and with New Zealand — both through articles in 'The Pacific Way' and through articles from technicians in the Pacific that can be published in this Journal — these I know would be greatly appreciated by New Zealand and other Pacific laboratory workers.

Overseas Aid Committee

John Elliot on behalf of the Overseas Aid Committee recently wrote to previous students of the P.P.T.C. and sent them complimentary copies of the N.Z.I.M.L.T. Journal. As a result of this correspondence the following letters have been received:

From C.W.M. Hospital, Suva.

"Dear Mr Elliot,

Your name and address was provided to me by one of my colleagues Mrs Sainimere Bavoro who came to Wellington on a training course sometimes ago.

I would be quite keen on publishing some papers or other interesting material from our microbiology unit.

I shall be grateful if you may provide more information about your journal and nature of papers you routinely publish.

(Signed) R. Parmar."

From Tarawa, Kiribati

"Dear John Elliot,

This is in answer of your letter dated 21st Dec., 1983 which I received late on 10th Jan., 1984.

It is very interesting to notice about the New Zealand Institute of Medical Laboratory Technology in trying to keep in touch with other small laboratories.

When I left the Pacific Paramedical Training Centre I still thought of ways which I could communicate with its people. Sometimes I wrote letters to the Centre (P.P.T.C.) to find some informations or asked questions which still confused me during the course. I thanked Andrea Hall for her willingness to communicate and in sending me everything I asked for.

I wish to let you know that I am willing to contribute, but next time I receive your Journal, I want to get informations on articles and informations which can be published.

(Signed) Baibuke Tauro."

From Port Moresby General Hospital, Papua New Guinea.

"Dear Sir,

I thank you on behalf of students who attended that Pacific Paramedical Training Centre of the continued link in that respect. We are happy to contribute when something of great interest arises in this ever expanding field of Science from our end.

We shall be very thankful if you continue to send us copies of your "Journal" everytime one is available.

(Signed) Saleu Kutan".

The editor of the Journal on behalf of the N.Z.I.M.L.T. will continue to send complimentary copies of the Journal to Pacific Island Laboratories.

LETTERS TO THE EDITOR

National Immunohaematology Proficiency Survey (NIPS)

Dear Sir,

Laboratory proficiency testing surveys undoubtedly serve a beneficial purpose in providing opportunities both for education and to provide insight into deficiencies in the quality of test performance. An adverse effect that appears to be universal, however, is that subsequent published discussion by those appointed to set the survey exercises and to analyze the responses sometimes has a tendency to create distorted perceptions concerning the qualities that are to be considered desirable in commercial laboratory reagents. Such comments carry special authority because their source is deemed to be divinely inspired and infallible, thereby imposing pressure on manufacturers to meet imagined requirements that are not only unreasonable but also impracticable.

A case in point, perhaps, is the summary of results obtained in the National Immunohaematology Proficiency Survey (NIPS) from December 1982 through August 1983, which was written by Austin and Knight on behalf of the Technical Subcommittee of the Transfusion Advisory Committee and was published in the December 1983 issue of the *Journal*[1]. The summary in question, though admirably detailed and inviting willing concurrence in most of its conclusions, does nevertheless appear to place undue emphasis on the importance of blood group antibodies that are to be detected only by the application of an enzyme test procedure. In particular, the implication that an example of anti-C^w detectable only in an enzyme test is necessarily to be considered clinically significant is at least debatable, and the assertion that it is a recommendation by the Office of Biologics Research and Review (formerly the Bureau of Biologics) that Reagent Red Blood Cells used for antibody screening should include a C^w+ cell suspension is factually incorrect. Such a suggestion was indeed included among others presented by the Blood Products Advisory Committee for *consideration* by the Bureau of Biologics, but the charge of the Advistory Committee was to consider amendments to "Additional Requirements for Reagent Red Blood Cells" in U.S. Federal Regulations that might be applicable should manufacturers choose to recommend their products for use in antibody screening when no antiglobulin crossmatch would be performed at all. As things turned out, the reagent manufacturers unanimously declined to advocate the substitution of antibody screening for crossmatching, and the Advisory Committee's recommendation was accordingly never officially adopted.

The meeting of which the proceedings were reported by Garratty[2] (cited by Austin and Knight in their summary) was in fact called to exchange views on the many aspects to be considered should the antiglobulin crossmatch cease to be a mandatory requirement, and among the subjects discussed was the sheer impracticability of expecting manufacturers consistently to supply pairs of screening cells that possess homozygous expression of several antigens and also include C^w.

Abundant and convincing data were presented at the meeting that the risk of omitting the antiglobulin crossmatch is infinitesimal once the recipient's serum has been screened for unexpected antibodies by the indirect antiglobulin test and found to yield a negative result. Since these data were accumulated using commercial Reagent Red Blood Cells that did not invariably possess homozygous expression of selected antigens, nor invariably include C^w, the same data suggest that there is little need to clamour for stricter requirements to be imposed upon commercial Reagent Red Blood Cells. There is no denying, of course, the homozygous expression of certain antigens contributes to the sensitivity of the antibody detection test, but if this necessitates screening against three cells instead of two the advantage of deleting the antiglobulin crossmatch is diminished, particularly when there are other variables influencing sensitivity that lend themselves to optimal adjustment with greater ease.

As to the question of whether or not it is essential to apply an enzyme test procedure in detecting antibodies, one would have to consider what evidence there is to suggest that serious adverse consequences have resulted from failure to do so, rather than forming a conclusion based on the supposition that a given antibody is necessarily of clinical significance in all cases. Anti-C^w is, to be sure, not all that uncommon as a specificity detected only by an enzyme technique, more often than not in persons not known to have been exposed to a red cell-induced stimulus. Yet it would be difficult to find in the literature a single report to indicate that failure to detect this antibody had resulted in a significant haemolytic transfusion reaction, even though an overwhelming majority of antibody detection tests performed in the United States, both for screening and crossmatching, omit any kind of enzyme test procedure. Hardly anyone on this side of the Pacific Ocean would have found the incompatibility due to anti-C^w in Serum 090, but it is hard to see this as "a problem that hopefully will be resolved," in light of the enormous number of transfusions administered in this country and the projected frequency with which C^w+ blood must be administered to recipients whose serum contains undetected anti-C^w.

Yours sincerely,

John Case, FIMLS
Director of Regulatory Affairs
Gamma Biologicals, Inc.
Houston, Texas 77092

References:

1. Austin, R.J., Knight, A.E. National Immunohaematology Proficiency Survey (NIPS): A summary of results. *N.Z.J. Med. Lab. Tech.* 1983; **37** 117-118.
2. Garratty, G. The role of compatibility tests. *Transfusion* 1982; **22:**169-172.

Course 2 Allergy Update.
The Auckland Meeting has been transferred from the 30th June to the 1st September 1984, Auckland School of Medical Technology.

POETS CORNER

Photographers at North Shore

Me and Sid, see, we work for the paper,
Takin' photos of all the hot news;
(Like Piggy's old eight-dollar caper,
And the carnage from driving with booze.)

So the boss said to me and to Sid, here,
"Hey lads, for you two I've a chore;
Take your cameras and flashbulbs and such gear
To the Hospital, lads, on the Shore."

He muttered to us, "Aye, I hear tell
The place is wood-panelled, and plush,
Like a stay in a bloody great hotel —
It's enough to make taxpayers blush!"

So Sid and me went to take pictures
All over this Hospital base;
And the job went along without hitches,
Till we came to a really queer place —

Pathology Lab, folk were sayin',
(Whatever that may mean to them),
And a really odd bloke were there playin',
In the part that they called "Biochem".

The thing that he played with had buttons
And numbers and lights; it were weird!
We couldn't hear words, only mutters
That he made as he tugged at his beard.

I don't know what language he spoke in,
I can't rightly say what he said,
Perhaps the machine had got broken,
Or the bloke wasn't right in the head.

I might have heard "profile selections",
And I think that he said "nineteen tests";
(Didn't like to ask too many questions,
Seein' how me and Sid was both guests).

So we took all our photos, with him still
Peerin' worriedly down at them lights;
I think someone said he'd be there till
He'd got that machine back to rights.

Then we left, Sid and me, without findin'
The function of that funny bloke —
(Though we know about Doctors, and mindin'
Of patients and other sick folk.)

The machines that these hospitals have now!
Like magic they are, right enough,
And the likes of us can't even see how
They do all this Chemistry stuff!

R. G. Cooper

A EULOGY TO COMPLEXITY

To be or not to be
A hepatitis laboratory
Is a question asked of us
But we never make a fuss.

To determine either HAV or HBV
Or whether Non A Non B
Maybe CMV or EBV
Or other strange entity.

So many tests we do have
like Austria and Ausab
HBe and PVC
Microplates U and V
And even a little Terasaki.

sAg and sAb
eAb and eAg
IgM or IgG
And subtype Y or D.

No more SID or CEP
Nor even CFT
But modern PHA and RIA
And even a coloured enzyme assay
They are really here to stay
Such an important part to play

Many animals to inject
Hepatitis to detect
Turkey, sheep and rabbit three,
Then horse, chicken and a chimpanzee

Many kits that must sell
Kits like Antihebsgencell
Ausria, Cordia, and Auszyme,
Auscell, Hepatab it's hard to rhyme,
Hepanostika and Raphadex,
Macrovue and Antigex

But of course and nonetheless
The cheap and useful Hepatest
Now Corab Corzyme and Havab M
When will it ever end
Corzyme M now comes along
For Hepatitis B it can't be wrong

So now with hands that are steady
Take your pipettes and make ready
Diversity of testings, have your fill,
Just be careful of the aerosol

For hepatitis affects the liver
Slowing the bilirubin to deliver,
All at once a yellow shade
then A.C.C. to be paid.

It really is a necessity
That we wash our hands frequently
We hope that one day we will find
Hepatitis left behind

To be or not to be
A viral hepatitis laboratory
All in all we say sincerely
Hepatitis must be tested clearly.

D.G. Woodfield
R.A.M. Anderson

Advanced Course in Animal Technology (Tec Higher Certificate/IAT Fellowship)

The Course

A high level course for suitably qualified workers in laboratory animal facilities. The course will offer instruction in aspects of laboratory animal science and technology including animal physiology, genetics,

nutrition, immunology, pathology, embryology, gnotobiotics, law and animal house management. The subject matter covered will allow participants to enter IAT Fellowship and TEC Higher Certificate examinations.

Course participants will also be able to obtain work experience at a major British laboratory animal facility during which they will have an opportunity to study at first hand the management and work of such a unit.

Location

The course will be based at North East Surrey College of Technology, a major British centre for further and higher education. The College is situated on an attractive campus approximately 28 Km to the south-west of London. Heathrow Airport is 28 Km away and Gatwick Airport 23 Km.

Duration

One year, full-time, commencing Monday 8th October 1984.

Entry Requirements

Associateship of the Institute of Animal Technicians or an equivalent qualification.

Language

The course will be run entirely in English.

Accommodation

This will be arranged by the College's Accommodation Office.

Fees

The total fees for the course are £3,400. Living and accommodation expenses will amount to a further sum of approximately £3,600. Applicants are advised to approach sponsoring agencies for funds as soon as possible.

Further Information

Full details of the course content and an application form may be obtained from: Dr. W.M. Kurowski, Department of Applied Science, North East Surrey College of Technology, Reigate Road, EWELL, Surrey KT17 3DS, England.

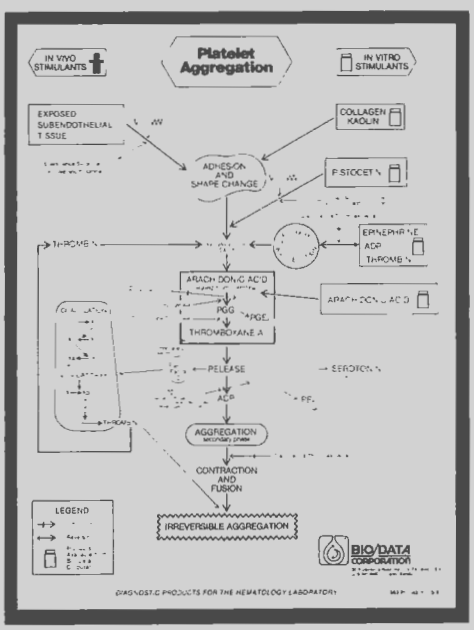
Answers to self-assessment questions Contaminant or Opportunist?

1. (a) *Bacteroides ureolyticus* vs *Eikenella corrodens*.
   (b) *E. corrodens* produces an hypochlorite odour.
   (c) Clindamycin/lincomycin and nitroimidazoles (metronidazole, tinidazole, ornidazole).
   (d) False. In 5 of 33 recent infections at Middlemore Hospital in which *E. corrodens* was involved, it was recovered in pure culture.
2. (a) False. In this situation *S. epidermidis* is commonly pathogenic. Recovery in pure culture in association with pus is good evidence of its pathogenicity in this instance.
   (b) False. Superinfection is unlikely to ensue so rapidly.
   (c) False. Mutation to resistance is exceptionally uncommon.
   (d) False. Removal of a foreign body is often necessary to achieve cure, especially with Gram negative or fungal infections. However, antibiotics alone are effective in many cases of infection due to Gram positive cocci and are well worth trying when the foreign body can not be readily or conveniently removed.
   (e) Methicillin-resistant strains of both *S. aureus* and *S. epidermidis* are 'heteroresistant' to penicillins and cephalosporins, i.e., there is a susceptible and resistant population in every culture. The resistant population grows more slowly except at low temperatures or in increased salt. Susceptibility testing should therefore incorporate one or more of the following:
      (i) incubation at 30-35°C
      (ii) incubation for 48 hours
      (iii) increasing the salt concentration of the medium by adding 2-5% NaCl
      (iv) use of a heavy inoculum 10^7 — 10^8 cfu.
   (f) Vancomycin, rifampicin and fusidic acid. Cefamandole is commonly active *in vitro* taking all the above precautions, but unequivocal proof of its clinical efficacy is lacking.
3. (a) (v). Certain microorganisms, particularly *P. cepacia*, *E. cloacae* and *E. agglomerans* are recognized as causes of sepsis from contaminated infusate. An epidemic of septicaemia due to one of these should suggest this source even though the other routes are possible.
   (b) (iv) and (v). The organism is unlikely to be transmitted from patient to patient and closure of the unit is therefore inappropriate. It does not often colonise hospital personnel. Organisms in sinks seldom bear any relationship to those infecting patients.

Answers to Biochemical Calculations — Section II: Molecular Weights

1. 180
2. 60
3. 113
4. 40
5. 1 mole
6. 0.5 mole
7. 1 mmole
8. 0.25 mole
9. 1 mol/l
10. 1 mol/l
11. 0.5 mol/l
12. 5 mmol/l
13. 1 mmol/l
14. 5 mmol/l
15. 90 g
16. 20 g
17. 11.3 mg
18. 150 mg
19. 5.85 g
20. 272 g
21. 87 g
22. a) 50 mmol/l b) 100 mmol/l c) 50 mmol/l
23. a) 50 mmol/l b) 50 mmol/l c) 150 mmol/l
24. 9375 μmol/l (This result is very high compared with the reference range for serum iron — therefore methods for serum iron must not measure haemoglobin iron).
25. 735 μmol/l

NEW PRODUCTS AND SERVICES



FREE PLATELET AGGREGATION WALL CHART

Bio/Data International Corporation, Hatboro, PA, is pleased to offer, free of charge, a 17'' x 22'' wall chart which provides a schematic overview of platelet aggregation.

The colorful chart illustrates the process of platelet aggregation from shape change and adhesion through to irreversible aggregation. Highlighted are key areas such as the role of aspirin in the inhibition of

prostaglandin synthesis and the interaction of the coagulation and aggregation pathways.

The chart displays the various disease states which affect platelet function at their point of interference. Additionally, the mode of action of in vitro aggregation stimulants is demonstrated, providing a useful reference for interpretation of laboratory test results.

The platelet aggregation chart is a highly useful instructional aid for clinicians, laboratorians, and students.

To obtain a free copy of the PLATELET AGGREGATION chart, contact BIO/DATA INTERNATIONAL CORPORATION, 3615 Davisville Road, Hatboro, PA, 19040, U.S.A., or **Circle 31 on the readers reply card.**

BECKMAN HPLC COLUMNS
FOR PURIFICATION OF PROTEINS

Beckman Instruments, Inc. has introduced several new preparative 21.1 mm (1'') ID Spherogel™ TSK-SWG columns for high performance liquid chromatographs. The columns are designed for large scale purification of proteins. More than 350 mg of BSA has been purified by these new columns.

Columns come in 30- and 60-cm lengths in 2,000, 3,000 and 4,000 SW packing materials. To extend column life, pre-column and column repair material is available.

For more information contact Alphatech, Phone 770-392 Auckland, or **Circle 42 on the readers reply card.**

BECKMAN ANNOUNCES NEW DIAGNOSTIC
PRODUCT LINE

Beckman Instruments, Inc announces a comprehensive general-purpose clinical chemistry reagent product line from the newly formed Clinical Diagnostics Division of Carlsbad, California, USA.

Reagents for 27 chemistries are available either in dry formulations under the Dri-STAT™ name, or liquid formulations under the Liquid-STAT™ name. The product line includes SCE (Scandinavian Committee on Enzymes) formulations for ALT, AST, CK-NAC and LD-P and rate optimized methods for BUN and Triglycerides. Reagents are packaged in tablets, single and dual-vial forms, and most are stable for three years from the date of manufacture.

Beckman provides continuously updated applications data for use on a wide range of clinical instruments including centrifugal fast analyzers, flow cell spectrophotometers and rate reaction systems. Reagents are available for these chemistries:

Dri-STAT Chemistries

ACP	Chol-ES	PAP
ALP	Chol-HDL	Trig-UV Endpoint
ALP-DEA	CO_2	Trig-INT
ALT	CK-NAC	Uric Acid Trinder
ALT SCE	CK	Uric Acid-UV
Ammonia	CK-MB	
Amylase-DS	GGT	
AST	Glucose HK	
AST SCE	HBD	
BUN Rate	LD-L	
BUN Endpoint	LDP SCE	

Liquid-STAT Chemistries

Albumin	Calcium	Iron/IBC
ALP	Cholesterol	LD-L
ALT	CK	Phosphorus
AST	Creatinine	Total Protein
Bilirubin	Glucose	

Information on applications for any Dri-STAT or Liquid-STAT product is available from your local Beckman Sales and Service office or from authorized dealers worldwide. For more information, contact Alphatech, Phone 770-392, Auckland, or **Circle 40 on the readers reply card.**

LAB-LINE® ORBIT INCUBATOR-SHAKER

LAB-LINE INSTRUMENTS, INC. announces the release of a new Orbit Incubator-Shaker. This model, available in two sizes, combines hydraulic thermostat control and a dependable mechanical timer with variable shaking speed. Temperature range is from slightly above ambient to 60°C. The shaker can be operated continuously or timed for up to one hour while the shaking speed of 25-400 RPM is displayed on a direct reading tachometer.

Additional features include a conveniently located control panel, a viewing window allowing inspection of samples without lifting cover, a safety interlock, interrupting shaker action if cover is opened.

Options include a full line of shaker platforms, a locking clear control panel cover and factory installed cooling coils, gassing manifolds and light bank.

For unsurpassed accuracy and operating ease, LAB-LINE also has available a microprocessor controlled model.

For further information contact Wiltons, P.O. Box 31-044, Lower Hutt, or **Circle 46 on readers reply card.**

STAT L-LACTATE ASSAYS OF WHOLE BLOOD
IN 45 SECONDS

The YSI Model 23L Lactate Analyzer measures L-lactate concentrations rapidly, with minimum sample preparation for acute care diagnostic use, exercise physiology, sports medicine and research applications. Samples can be whole blood, plasma or cerebrospinal fluid.

A sample is drawn from the patient and 25μl is injected into the Model 23L. Results are displayed 45 seconds later on an LED readout in millimoles per liter, permitting an analysis rate of 42 samples per hour. Instrument range is 0-15.0 mmol/1; sensitivity is 0.1 mmol/1. Samples above the upper limit may be assayed after dilution.

The Model 23L uses an enzymatic polarographic probe to detect hydrogen peroxide (H_2O_2). The enzyme, L-lactate oxidase, is immobilized between layers of polycarbonate and cellulose acetate. This trilaminated membrane, placed next to the probe, prevents substances normally found in blood from interfering with the enzyme or the probe. L-lactate, however, freely diffuses through the polycarbonate membrane where the enzyme converts it to H_2O_2. In turn, the H_2O_2 diffuses through the cellulose acetate layer to the probe, where it is measured amperometrically. The signal produced is directly proportional to the L-lactate in the sample.

For further information contact Wiltons, P.O. Box 31-044, Lower Hutt or **Circle 47 on readers reply card.**

COMPUDIL

We are very pleased to announce the introduction of the Compudil. This new instrument is primarily a DILUTER, but it can be used to perform a number of additional functions such as dispensing and titrating. The Compudil achieves its flexibility by full use of a specially designed microprocessor, which also allows a user to store up to 21 individually written programmes in its memory.

Our objective in developing this model is to expand our existing product range with an instrument using the most up-to-date microprocessor technology combined with the highest possible precision, so that all current major clinical assays can be undertaken with it.

For further information contact Wiltons, P.O. Box 31-044, Lower Hutt or **Circle 48 on readers reply card.**

VERSATILE FREEZE DRYER FITS EASILY ON COUNTER TOP

The VirTis 3 liter benchtop laboratory freeze-dryer is now available with either shell freezing bath ''built-in'', or low temperature cascade refrigeration. This new model is just 9 inches longer than the standard unit, yet it incorporates an extra 12½'' x 4½'' x 6½'' shell freezing bath for temperatures down to -45°C. It also includes a built in 90 liter per minute vacuum pump.

Alternatively, the increased size may be used to accommodate the vacuum pump plus a sealed, double compressor system for cascade refrigeration down to -85°C.

The 3 liter benchtop unit comes complete with a standard 12 port drum manifold (including quick seal valves), electric vacuum gauge, electric temperature gauge and all stainless steel work surface. The ''snap out'' sides offer ease of cleaning and servicing.

For further information contact Wiltons, P.O. Box 31-044, Lower Hutt or **Circle 49 on readers reply card.**

HEWLETT PACKARD FORMS NEW PC ORGANISATION
WITHIN ITS MEDICAL PRODUCTS GROUP

Hewlett Packard have recently announced the formation of a personal computer, medical marketing organisation within the HP Medical Products Group. It will market the HP 150 touchscreen personal computer and its medical application software to private practitioners, clinics and hospitals.

Introduced in September 1983, the HP 150 uses a touchscreen display and is designed to be the easiest personal computer (PC) for first time computer users to learn and operate.

Hewlett Packard has 25 years of experience in the manufacture of medical equipment, and is a leader in the manufacture of both computers

and medical instrumentation. Their experience has helped them identify the growing need for healthcare-oriented, personal computer products. Hewlett Packard understand the needs of the medical professional and have the products and service to help physicians, clinics and hospitals, make the inevitable step toward computerisation.

The new organisation will be responsible for handling all product management, development, sales and marketing efforts for the HP 150 in the healthcare field, as well as for the HP 150's medical application software. The PC organisation will have access to all the resources of the HP Medical Products Group, including its direct sales, service and support people.

For further information, contact the New Zealand distributors for Hewlett Packard Medical, Analytical and Personal Computer Products: Northrop Instruments & Systems Limited, P.O. Box 2406, Wellington, Telephone 856-658, P.O. Box 8602, Auckland, Telephone 794-091, P.O. Box 8388, Christchurch, Telephone 488-874, or **Circle 44 on readers reply card.**

BECKMAN CALIBRATION TEST ACCESSORY CHECKS UV/VIS SPECTROPHOTOMETERS

A calibration test accessory from Beckman Instruments, Inc. provides automatic performance checks for the company's DU®-6 and DU®-7 UV/Vis Spectrophotometers. It is especially useful in industries where self- or outside regulation is necessary.

The accessory does instrument performance verification, including numerical evaluation of photometric accuracy and repeatability, resolution, noise and percent stray light. There is no need to use liquid solutions for the performance checks. Hard copy of all data is provided.

For more information, contact Alphatech, Phone 770-392 or **Circle 45 on readers reply card.**

BOEHRINGER MANNHEIM INTRODUCES THE HITACHI 737

Based upon the acceptance of the 705 concept (with more than 1500 installations worldwide) the new HITACHI 737 has been developed for laboratories with a heavy workload. It is a discreet, selective, multi-analyzing system. With 23 chemistries including Na^+, K^+ and Cl^- by ion selective electrodes, it has a maximum output of 1200 tests/hour or 60 - 300 samples/hour. Employing either a one or two reagent system, end-point (with or without sample blank correction) kinetic and fixed time kinetics methods can be used. Reagent consumption is 350 μl/test (R^1=250 - 350 μl; R^2= 50 - 250 μl) and only 3 — 10 μl serum/test.

The sample tray uses plastic cups similar to the 705 and has the capacity of up to 60 positions for patient samples, QC's, standards and urgent specimens. A reaction disc containing 192 glass cuvettes with two washing systems means that there are no disposables. This disc is surrounded by a temperature controlled (variable from 25°C to 37°C ±0.1°C) circulating water bath. The spectrophotometer is a multiwavelength system ranging from 340 to 700 nm. Floppy discs are used for both working programs and patient demographics on the data processing unit.

Last of all, does it fit in your laboratory? It is 2.04 metres wide, 0.8 metres deep, 1.75 metres high and weighs in at 600 Kg.

Interested? For further information please contact your Boehringer Mannheim agents Smith-Biolab. Telephone Auckland 483-039, or **Circle 43 on readers reply card.**

NZIMLT Dunedin 1984

New Zealand Institute of Medical Laboratory Technology

40TH ANNUAL SCIENTIFIC MEETING

THEME —

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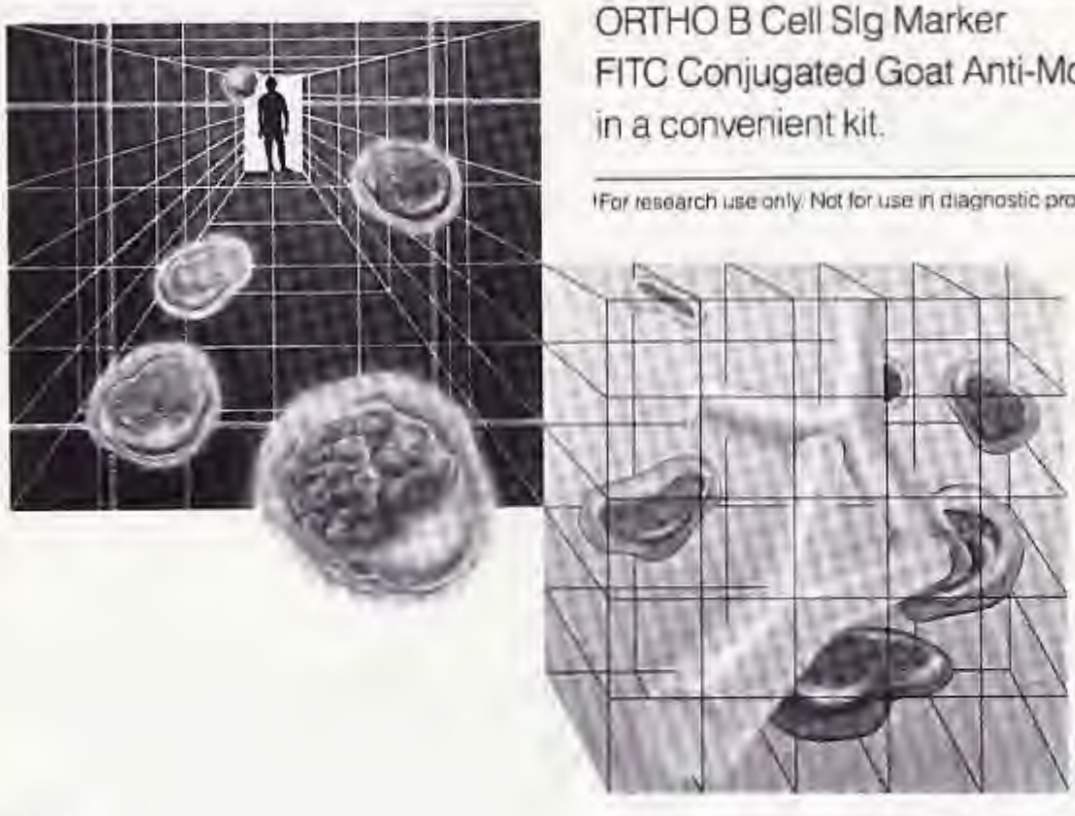

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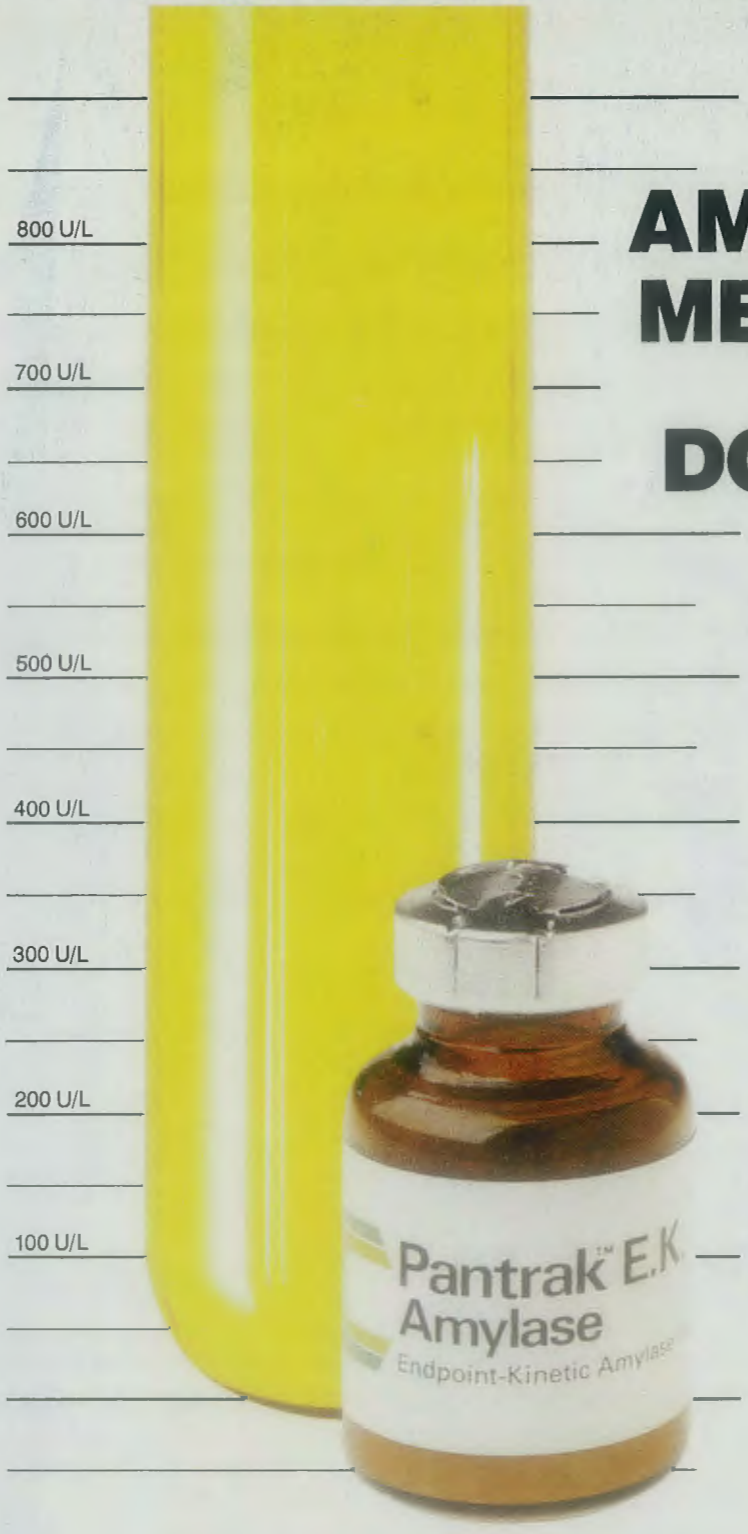

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