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LIMITED FUNCTION AUTOMATION IN CLINICAL CHEMISTRY

Ronald James Georges

A dissertation submitted to the Faculty of Medicine, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the Degree of Master of Science in Medicine.

Johannesburg
July 1981

I hereby declare that the dissertation "Limited Function Automation in Clinical Chemistry" submitted for the degree of Master of Science in Medicine in the University of the Witwatersrand, Johannesburg, is my own work and has not been submitted before for any degree or examination in any other University.

R.J. Georges.

Ronald James Georges.

July 1981.

Parts of this dissertation have been published in the following journal articles (Appendices 1 and 2):-

1) Problems associated with the large-scale centralisation of routine biochemical work.
Georges, R.J.
Medical Laboratory Technology, *28*, 191-196 (1971).

2) Micro-determination of serum alkaline phosphatase using phenolphthalein monophosphate as substrate.
Georges, R.J. and Persona, E.
South African Journal of Medical Laboratory Technology, *25*, 63-64 (1979).

THE COMMONER BIOCHEMICAL TESTS
AND
THEIR VALUE IN MEDICAL DIAGNOSIS.

Some brief Notes as to their Scope, with Instructions for the collection of suitable Specimens.

By F. William Fox, D.Sc. (Lond.),
Biochemical Department, South African Institute for Medical Research.

Biochemical tests are becoming indispensable for the accurate diagnosis of certain types of disease. By many, however, they are regarded as being complicated and suitable only for the specialist; but this is often far from being the case. The object of these notes is to call attention to some of those most widely used, and to indicate both their value and simplicity. From time to time new tests, which have been proved to be of value, will be adopted for routine purposes; particulars of such tests may be obtained on application.

(1)—BLOOD SUGAR.

The estimation of the amount of sugar in the blood has rapidly become of fundamental importance in the detection and control of diabetes.

After a night's fast, or from two to three hours after a meal, the percentage of sugar in the blood of a healthy person approximates closely to 0·1 gm. per 100 c.c. (range 0·07 to 0·11). The diabetic, however, is unable properly to deal with the sugar he consumes, and, consequently, the amount in the blood tends to

Opening page of Biochemical Department Handbook (July 1929).

Frontispiece

To F. William Fox,

Biochemist to the South African Institute for Medical Research, 1925-1953.

Pioneer.

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The production of the finished work has been dependent on the collaboration received from Mr M. Anderson of the Photographic Department, Miss S. Hall of the Department of Medical Illustration, Mrs L. Battaglia of the Central Institute Library, Mr H. Peyper of the Printing Department, and Mrs S.L. Woods who typed the manuscript. To all these persons I extend my grateful thanks.

LIST OF ABBREVIATIONS

ABA	Abbott Bichromatic Analyser
ACTH	Adrenocorticotrophic hormone
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AMP	2-Amino-2-methyl-1-propanol
AST	Aspartate transaminase
BCG	Bromocresol green
BUN	Blood urea nitrogen
CAP	College of American Pathologists
CHOD/PAP	Cholesterol oxidase/phenol, 4-aminophenazone
CK	Creatine kinase
CPC	Cresolphthalein complexone
CSL	Clinical Sciences and Manufacturing Laboratories (Pty) Ltd
CV	Coefficient of variation
DAM	Diacetyl monoxime
DEA	Diethanolamine
EDTA	Ethylenediamine-tetraacetic acid
EEL	Evans Electroselenium Ltd
EGTA	Ethyleneglycol bis (2-aminoethyl ether) tetraacetic acid
G6PD	Glucose-6-phosphate dehydrogenase
GLDH	Glutamate dehydrogenase
GO/PAP	Glucose oxidase/phenol, 4-aminophenazone

IFCC	International Federation of Clinical Chemistry
IL	Instrumentation Laboratory, Incorporated
LB	Liebermann-Burchard
LD	Lactate dehydrogenase
LED	Light-emitting diode
MSC	Multi-System Control Serum
N/S	Non-significant
OD	Optical density
P	Probability
PNPP	p-Nitrophenyl phosphate
QC	Quality control
r	Correlation coefficient
SD	Standard deviation
SMA	Sequential multiple analyser
SMI	Scientific Manufacturing Industries
TQC	Technicon Quality Control Serum
UV	Ultraviolet

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SUMMARY

In this dissertation a case is put forward for the use of work-simplified methods, based on the mechanisation of certain key-stages only in the analytical process ("limited function automation") as an alternative to the use of complex and costly large analysers for the performance of quantitative clinical chemical assays.

The introductory chapters examine the evolution of clinical chemistry in the environment of the hospital service laboratory, with particular reference to the growth in the number and variety of diagnostic investigations carried out. Developments in methodology and instrumentation making possible the partial or total mechanisation of analytical processes are discussed and problems associated with the large-scale centralisation of routine biochemical work in the "factory laboratory" are considered.

The practical objective of this work was the development of standardised methods, based on limited function automation, for introduction into some fifty of the Branch Laboratories comprising the Regional Laboratory Service operated by the South African Institute for Medical Research. A preliminary survey identified, for six representative laboratories, the eighteen most commonly performed quantitative tests, from which a group of twelve were selected for study. Choice of methods was made from analytical performance data contained in the six-monthly end-of-cycle surveys of the Wellcome Quality Control Programme, taking into account previous investment in standardised instrumentation in the Branch

Laboratories.

The assays and methods investigated were: sodium and potassium (IL 343 Flame Photometer); chloride (Radiometer CMT-10 chloride meter); bicarbonate (Harleco micro gasometer); urea (direct diacetyl monoxime); creatinine (Jaffé reaction with deproteinisation); glucose (GO/PAP); albumin (BCG dye-binding); calcium (cresolphthalein complexone); cholesterol (CHOD/PAP); alkaline phosphatase (phenolphthalein monophosphate) and aspartate transaminase (rate-reaction at 340 nm).

An early trial of nine of these assay-procedures (omitting bicarbonate and the two enzymes) during one cycle of the Wellcome Programme showed their between-batch analytical precision to equal or better that for "all methods" and for the specific method itself, for all analytes other than glucose. In the latter case a single discrepant result gave rise to an inferior SD of 0,40 mmol/l. Concurrently with the above survey the performance characteristics of several semi-automated sampling and measuring devices used in the work-simplified techniques were assessed by gravimetric, photometric and radioisotopic techniques. The operational coefficients of variation of the Oxford Samplers, Pipettors and Dilutor, SMI Micro/Pettor, Clay-Adams Selectapette and the Gilford 300-T spectrophotometer ranged from 0,11% to 1,56%, while sampling carry-over with the 10 µl and 20 µl SMI Micro/Pettors was under 0,1%.

For all twelve analytes a study to establish well-population reference values, in parallel with the Technicon SMA 6/60 and 12/60,

was carried out on twenty male and twenty female blood donors. Good agreement with previously established reference values was found apart from those for urea, glucose and cholesterol where wider ranges were encountered; in the latter two instances this was largely due to the existence of single high outlying values.

The setting up of an Interlaboratory Quality Control Programme for SAIMR Laboratories in 1980, similar in operation to the National Quality Control Scheme established in the UK in 1969, provided an opportunity for a "field trial" of the proposed standard methods. The average inter-laboratory CV for the seven controls issued during 1980, for the ten assays common to the SAIMR and UK Programmes ranged from 1,36% for sodium (IL Flame Photometer) to 17,6% for alkaline phosphatase ("Phosphastrate"). The average CV for all ten analytes was 8,27%, compared to 8,59% for overall methods (manual and automated) in the UK Scheme during the period 1969 - 1971.

In the penultimate chapter, the analytical precision of the proposed work-simplified methods, as assessed in a number of the foregoing studies, is set out against some of the widely divergent published criteria on "performance requirements for medical usefulness" for quantitative clinical laboratory assays. The short-term between-batch analytical CV was less than half of the value for composite biological CV published by Young in 1970, in all cases apart from glucose ("normal range" values) and calcium.

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CHAPTER 1

1. THE SCOPE AND HISTORICAL DEVELOPMENT OF CLINICAL CHEMISTRY

1.1 Introduction

Clinical chemistry has been defined as:[1]

> "The study of the chemical aspects of human life in health and illness and the application of chemical laboratory methods to diagnosis, control of treatment and prevention of disease."

In the manner of its wording this definition recognises a basic division of the subject into two overlapping and mutually supporting components:

a) Fundamental research into the chemical mechanisms of disease and on laboratory methods for their elucidation.

b) A "service" function, concerned with the investigation of individual patients by means of tests and analyses made available in hospital pathology departments.

While the service component is dependent on the "research" component for indicating which investigations may be useful and for providing methods for carrying them out, its scale of activity in terms of numbers of persons involved and operating costs is by far the larger of the two.[2] Furthermore, the hospital routine laboratory is the principal point of contact between clinical chemistry and the population at large.

The methods of clinical chemistry have been applied to medicine only since the beginning of this century, initially on a small scale but lately on a very large scale and in almost

every field of medicine.[3] The two main reasons for the performance of chemical laboratory tests are:[4]

1) To help the clinician establish a diagnosis on which a rational approach to treatment and prognosis can be based.

2) In the control of treatment, by monitoring the changes in concentration of body constituents brought about by various therapeutic measures adopted (e.g. treatment with insulin; haemodialysis), including the monitoring of the circulating level of drugs themselves.

Various classifications of clinical chemical tests have been proposed. Eastham and Pollard[5] distinguished:

a) Diagnostic tests. Those which strongly suggest a particular diagnosis; for example, the finding of excessive amounts of homogentisic acid in the urine of homozygous alkaptonurics.

b) Tests supporting a clinical diagnosis; for example, an increased level of serum aspartate transaminase in a suspected case of cardiac infarction.

c) Non-specific tests, affected by the disease or its consequences; for example a raised serum cholesterol level in hypothyroidism.

Of a more fundamental nature with regard to the pattern of laboratory utilisation is the classification of Marks,[4] who distinguishes:

a) Single discretionary tests, specifically ordered by the clinician and designed, or intended, to answer specific questions, e.g. "Does this patient have a raised serum thyroxine concentration (supporting my tentative diagnosis

of hyperthyroidism)?"

b) Biochemical "profiles", where the laboratory provides values for two or more interdependent substances simultaneously in response to a single request. Examples of such profiles are "urea and electrolytes"; "acid base studies" (blood pH, PCO_2 and standard bicarbonate) and various combinations of "liver function tests". The use of such groupings of tests stems from the fact that often consideration of the set of results collectively provides more clinically useful information than any one of them alone, or all of them on different occasions.

c) Dynamic function tests, involving measuring the changes in the appropriate body fluid chemistry brought about by some standardised stimulus; for example, the extent and duration of hyperglycaemia following oral ingestion of glucose.

d) Screening tests. This last class of laboratory investigations can be further divided into:

   i) Hospital admission screening, in effect a set of biochemical profiles, encompassing around 12 - 20 tests, carried out on every patient admitted as an extension of conventional physical examination and simple side-room testing of the urine.

   ii) Well-population screening, concerned with the pre-symptomatic diagnosis of real or potential disease. Included here are neonatal screening for inborn errors of metabolism, screening "batteries" performed as part of a periodic health check-up and the screening of persons at risk through occupational exposure to toxic agents such as lead.

In respect of hospital patients a distinction can therefore be made between discretionary tests, ordered by the attendant clinician as part of the progressive diagnostic process (groups a - c) and screening tests, provided *en bloc* in order to initiate the diagnostic process where clinical symptoms may be unobserved or absent (group d).

The performance of a screening battery for the principal purpose of laboratory convenience will be discussed in the next chapter.

In summary, a definition of the service function of a clinical chemistry department has been given by Carter *et al.*[6] who consider that this should provide ...

> "in the most efficient, effective and economic way information which may help to make a diagnosis, may be needed to record objectively a diagnosis which may later be obscured by treatment, may indicate treatment and may be used to evaluate treatment. In other words, clinical chemistry should provide the clinician with adequate information for him to take useful decisions."

The points emphasised in the above definition are fundamental to the subject of this dissertation and will be considered further in later chapters.

1.2 Development of the service function of clinical chemistry at The South African Institute for Medical Research

Examination of the records regarding the variety and total numbers

of tests performed in an individual laboratory can provide a useful framework for studying the evolution of clinical chemistry.[7] The South African Institute for Medical Research, which began operating in 1914, encompasses all the main events of this evolution within its lifetime.[8]

From the time of the first Annual Report of the Institute in 1919 until 1952 essentially the same system of recording workloads was in use. Subsequently, fragmentation of the single Biochemical Department into a number of separate Units and a change-over to the recording of "work units" in place of actual test numbers makes it more difficult to extract information, but the test-frequencies for the year 1977 have been calculated.

The total numbers and types of tests performed in the years 1929, 1952 and 1977 are shown in Table I.[9,10,11]

During the first decade of its existence the Institute had no separate Biochemical Department and figures for the biochemical workload were included with the overall figures for all investigations. The latter increased from 9 091 in 1914 to 35 137 in 1923, in which year it was noted in the Annual Report:

> "Chemical examinations have increased in number largely through examinations of blood and urine called for in connection with diabetes and renal diseases. Blood sugar estimations reached 757 in number, this being largely accounted for, of course, by the use of insulin in treatment of diabetes."

The following year a Biochemical Department was established under the direction of Dr F.W. Fox and regular records of this

TABLE 1. Growth in routine biochemical workloads at The South African Institute for Medical Research, 1929 - 1977

VARIETY OF QUANTITATIVE ASSAYS PERFORMED: 1929	1929	1952	1977
BLOOD (urea, glucose, icteric index, chloride, alkali reserve, calcium, uric acid, cholesterol)	8	41	117
URINE (urea, creatinine, amylase)	3	22	28
CSF (glucose, chloride)	2	4	5
FAECES (split and total fat)	1	3	5
GASTRIC ANALYSIS (fractional test meal)	1	1	1
OXYGEN CONSUMPTION (basal metabolic rate)	1	1	-
JEJUNAL JUICE	-	-	3
SALIVA	-	-	4
SWEAT	-	-	1
VARIETY OF TESTS	16	72	164
PROPORTIONAL INCREASE	1	4,5	10,25
TOTAL NUMBER OF TESTS (QUANTITATIVE AND QUALITATIVE)	5 972	124 264	1 496 212
PROPORTIONAL INCREASE	1	20,8	250,5

Department's work were thenceforward kept. Test numbers increased from 4 241 in 1925 to 5 972 in 1929, in which year the Departmental Handbook illustrated in the frontispiece was brought out.

At the commencement of the 1930's blood glucose and urea and qualitative urine tests made up the bulk of the work. Other blood tests were generally relegated to a "miscellaneous" grouping in the Report, which grouping comprised in 1930 (tests per annum!):

Icteric index	110
Calcium	14
Uric acid	6
Cholesterol	3
Chloride	5
Alkali reserve	2
Spectroscopy	5

During the remainder of the decade before the Second World War the Department continued to expand slowly, the test number reaching 10 211 in 1939. Among additions to the repertoire were alkaline phosphatase, inorganic phosphate, plasma proteins and non-protein nitrogen (all in 1932); blood pH (1933), urine ascorbic acid (1936) and blood sulphonamides (1939). The galactose tolerance test of liver function was introduced in 1934, replacing the laevulose tolerance test which, remarkably, had been in use since before 1930. In both cases the blood level of "total reducing substances" rather than the specific sugar itself was measured.

Among various observations made in the Annual Reports of that period, two are particularly worthy of note as indications of sentiments which are with us to this day. Commenting on the

performance of about ten blood pH estimations, all of which were "normal", it was remarked in 1933:

> "We desire to record our conviction in this connection that abnormal results in these tests are not to be expected in patients who are able to walk in comfort to the laboratory."

The receipt of 178 blood specimens for tests under the "miscellaneous" heading in 1935 evoked the comment:

> "This group of analyses is more troublesome than the numbers imply, since they generally arrive singly, thus taking the maximum amount of time; are sometimes incorrectly collected, necessitating asking for another specimen and, in the case of the less common tests, require fresh preparation of reagents and standards for each occasion."

Whereas the war years were associated with a slowing-down or even reversal of the growth of workloads in many laboratories[3,7] a large influx of specimens from military hospitals augumented the Institute's turnover of tests between 1940 and 1942. Although this source later fell away the total of tests in 1945 (24 659) was well over double the last pre-war figure.

In 1947 it was noted that nearly 50 out of the 80 different tests performed were quantitative in nature and that the number of analyses in the miscellaneous group had grown from around two thousand in 1944 to six thousand in 1947. Increases in workloads and the introduction of new tests in the early post-war period continued as follows:-

1946 - 32 349

1947 - 40 007

1948 - 59 053 Chemical methods for sodium and potassium, 17-oxosteroids in urine, biuret method for proteins.

1949 - 75 836

1950 - 88 585

In 1950 another great increase in miscellaneous blood tests was ascribed to ... "the availability of advance supplies of ACTH and cortisone and numerous investigations of electrolytes and other blood constituents in patients receiving treatment therefrom."

1951 - 108 439 Paper electrophoresis.

1952 - 124 264 Flame photometry for sodium and potassium.

While detailed workload figures become inaccessible after 1952, the effect of the introduction of the flame photometer was sufficiently dramatic to warrant special mention in the Report for 1954:

Tests per annum	1952	1953	1954
Potassium	1 782	3 860	5 637
Sodium, chloride, CO_2 (combined total)	3 454	8 428	12 317

The introduction of new tests continued unabated during the decade from 1952 to 1962:

1952 - 53 Protein-bound iodine, Evans Blue blood volume studies, iron and iron-binding capacity, magnesium, bio-assay of urinary catecholamines, pregnanediol, follicle stimulating hormone, urinary corticosteroids.

1954	Vitamin A absorption test.
1955	Blood ammonia, urinary 17 oxogenic steroids.
1957	5-hydroxyindolyl acetic acid, urinary copper.
1958	Serum alanine- and aspartate transaminases.
1960	Xylose, phosphoethanolamine, phosphorus excretion index.
1961	4-hydroxy 3-methoxy mandelic acid.
1962	Caeruloplasmin, phenylalanine.

The last part of this decade saw the introduction of the single-channel Technicon Autoanalyser[12] for urea and glucose measurement, thus introducing the modern era of clinical chemistry. This event serves to lead into the next section of this chapter, where the development of clinical chemistry in respect of analytical methods and instrumentation will be further discussed.

1.3 Developments in instrumentation and methodology

Concern over the continuously increasing workloads in clinical laboratories is not a recent phenomenon. As long ago as 1923 the Annual Report of The South African Institute for Medical Research, after recording that the work of the Routine Division had doubled in each of the quinquennial periods since 1914, went on to observe:

> "It is to be hoped that this rate of increase will not continue for, if it be true as stated in Ecclesiasticus[13] that ... 'the wisdom of a learned man cometh by opportunity of leisure and he that hath little business shall become wise' ... then there is little hope of our officers

> acquiring wisdom, for even at present the opportunities for leisure have diminished almost to vanishing point."

It is frequently suggested that the relatively recent advances in technology, leading to the use of high-output mechanisation in a factory-like environment, are principally responsible for the situation in the clinical chemistry laboratory today - emphatically described by Potgieter:[14]

> "The tremendous technological advances especially since 1957 with the introduction of automated equipment have revolutionised the situation, certainly in chemical pathology. However, it also created a monster in terms of production of data which the laboratory could not handle using conventional techniques. The chemical pathologist suddenly had to become a machine-minder, a pusher of buttons, a computer expert, a systems analyst. The patient became a depersonalised check-digit indentity number. Better results were produced but they meant nothing to the pathologist because he seldom knew what was wrong with the patient or what effect treatment had. The only contact he retained with the clinicians was a regular monthly fight at staff meetings over poorly identified request forms, unlabelled specimens and incompletely collected 24-hour urines."

However, examination of the laboratory records (Table I) shows that this situation was on the way to developing before the first mechanised equipment had made its impact on the laboratory.

The overall rate of growth over 48 years from 5 972 tests performed in 1929 to 1 496 212 in 1977 represents a compounded

annual increase of 12,2%. During the approximate first half of this period (1929 - 52) the rate of increase was 14,1% and during the second half (1952 - 77) it was 10,5%. The astronomically large figures now being reached by this exponential growth tend to obscure the fact that the rate of growth was somewhat less in the later compared with the earlier years.

Furthermore, it is apparent that a greater proportional increase in the variety of tests occurred in the first period (16 —> 72) than in the second (72 —> 164).

These figures need to be interpreted with caution, for several reasons. The early growth rate was not constant; the near-doubling of the workload from 1946 to 1948 required the whole of the decade 1929 - 1939 for a similar proportional increase. Where only a small repertoire of tests was being performed, the number of new tests required to double this was correspondingly less. Finally, mechanisation has principally affected the throughput of quantitative tests, while the workload figures include both quantitative and qualitative ones. Nevertheless it seems fair to state that the present high level of laboratory utilisation had its origins in events which preceded the introduction of mechanisation.

A number of factors contributed to the pronounced upward spurt in the early post-war period. Principal among these was the realisation that the rigorously exact methods of quantitative analysis propounded some fifteen years earlier by Peters and Van Slyke[15] were unnecessarily complex and time-consuming for clinical use in many cases. Awareness of the extent of biological variation

in clinical chemical parameters, the need for speedier results inherited from the emergency situations of wartime and the frequent requirement for multiple analyses on a single specimen of blood led to the introduction of simplified semi-micro methods for many routine analyses.[16]

By way of example, it was found convenient and reliable to estimate the ammonia, formed from blood urea by the action of urease, colorimetrically with Nessler's reagent in place of aeration and titration. In the process, the specimen requirement was reduced from 3,0 ml to 0,2 ml. Similarly, the biuret method replaced the technically demanding Kjeldahl digestion procedure for proteins, and total cholesterol determination dispensed with hydrolysis of the esterified fraction. The adoption of many of these simplified methods was facilitated by the widespread introduction of the photo-electric colorimeter, described by Varley as "the most important single development in clinical chemistry".[7]

The introduction of flame photometry at the SAIMR has previously been mentioned. Writing in 1962, Eaton and Lathe[17] observed:

> "Fifteen years ago a single potassium determination required about an hour's work spread over the better part of 20 hours and it had an error of about 10%. Within 5 years the flame photometer had increased this speed about a thousandfold, the work required had been reduced to a fiftieth and the error to a third."

Concurrently with the introduction and great subsequent growth of mechanisation, further improvements and simplifications in the underlying physico-chemical principles of analytical methods have continued up to the present time. An indication of the changes in methodology, compared with a half-century ago, in respect of ten of the analyses chosen for investigation in this dissertation is given in Table II. Amidst all the other changes it is noteworthy that for the determination of bicarbonate and creatinine no fundamental change in methodology (liberation of CO_2 by acid; Jaffé reaction) has found its way into routine manual practice during half a century!

During the first post-war decade it was possible, therefore, for the laboratory to keep abreast of the rising number of requests by adopting some or all of the following measures:-[18]

1) Use of simplified, time-shortened methods.

2) Employment of additional staff.

3) Expansion into larger laboratory space.

It is not unreasonable to believe that, had the facilities of space and staff been unlimited, workloads might have grown to much their present levels even in the absence of more elaborate mechanical aids. However, the strain on financial resources posed by salaries and building costs was beginning to be felt by the year 1957 when an entirely novel solution was put forward by Leonard Skeggs:[12]

> "Many industrial laboratories, faced with similar increases in their work load, have derived benefit from the adoption of

TABLE II. The evolution of biochemical analytical methods in fifty years (1932 - 1981)

ASSAY	PETERS AND VAN SLYKE (1932)		USED IN THIS WORK (1981)	
	METHOD	SPECIMEN VOLUME	METHOD	SPECIMEN VOLUME
SODIUM	Gravimetric, after precipitation as sodium zinc uranyl acetate	1,0 ml	Flame photometry	0,1 ml
POTASSIUM	Colorimetric or volumetric after precipitation as potassium sodium cobaltinitrite	1,0 ml	Flame photometry	
CHLORIDE	Volumetric, iodate liberated from silver iodate	1,0 ml	Coulometric titration	0,02 ml
BICARBONATE	Manometric gasometric	0,2 ml	Volumetric gasometric	0,1 ml
UREA	Urease/aeration with titration	3,0 ml	Direct colorimetric (diacetyl monoxime)	0,01 ml
CREATININE	Jaffé reaction after deproteinisation, colorimetric	1,0 ml	Jaffé reaction after deproteinisation, colorimetric	0,5 ml
GLUCOSE	Copper-reduction/molybdic acid, colorimetric	0,2 ml	Enzymatic colorimetric, glucose oxidase	0,02 ml
ALBUMIN	Salt fractionation and Kjeldahl	0,5 ml	Dye binding with Bromocresol green	0,01 ml
CALCIUM	Oxalate precipitation, volumetric	2,0 ml	Colorimetric, cresolphthalein	0,02 ml
CHOLESTEROL	Liebermann-Burchard colorimetric, after solvent extraction and saponification	1,0 ml	Enzymatic colorimetric, cholesterol oxidase	0,02 ml

> instrumental and semi-automatic methods of analysis. It seemed worthwhile, therefore, to explore the possibility of automatic methods for analysis in clinical laboratories."

The results of this exploration and the impact which it has had on the clinical chemical laboratory form the topic for the next chapter.

CHAPTER 2

2. SYSTEMS OF TOTAL AUTOMATION AND THEIR IMPACT UPON THE LABORATORY

2.1 The automation of clinical chemical analyses

In its correct sense the term "automation" implies the replacement of human manipulative effort in the performance of a given process by mechanical and instrumental devices which are regulated by feed-back of information, so that the apparatus is self-monitoring or self-adjusting.[19] Since most of the devices used in clinical laboratories have no such feed-back control the term automation in this context is a misnomer and is recently being replaced by "mechanisation". However, as the earlier literature on the topic did not consider such a distinction the term automation is still widely employed by clinical chemists and consequently is used in the title and body of this dissertation.

As indicated in the previous chapter, the introduction of the photoelectric colorimeter in the 1940's led to the adoption of colorimetric methods for a wide range of routine quantitative assays in the clinical chemistry laboratory. Not surprisingly, therefore, it was for such procedures that the earliest fully-automated system was developed.[12]

The basic operations in colorimetric analysis fall into two groups:[20]

1) Unskilled operations, for example the setting out of test tubes and their transfer to and from a centrifuge, water bath or colorimeter.

2) Skilled operations involving measurement or calculation:

a) Measurement of specimens, filtrates, etc., usually in the volume range of 0,01 - 10,0 ml.

b) Absorbance readings on solutions derived from tests, standards and blanks.

c) Calculation of the numerical result for each individual test from the expression

$$\frac{\text{absorbance of test}}{\text{absorbance of standard}} \times \text{concentration of standard.}$$

The various handling operations in group 1 do not generally exert a serious rate-limiting influence on the output of work. In contrast, the measuring operations in the second group are inherently more time-consuming and are the steps at which errors, due to lack of skill, fatigue or boredom are most likely to take place.

All systems of automation are particularly directed towards the operations in the second group, the objective being that the newcomer to laboratory work should, with their aid, be able to produce results as rapidly and reliably as the trained and experienced technologist. The principal difference between total automation and limited function automation[21] is that the former includes mechanisation of the transport operations in group 1, so that no human intervention is required from the time the sample is automatically taken up from a turn-table until the final result is automatically recorded. In limited function automation, on the other hand, only those parts of the analytical process which are the most tedious and potentially imprecise are mechanised.

When exploring the possibility of automated analysis Skeggs[12]

considered that a robot-like device capable of imitating all the manual steps outlined above would be impracticable. Instead, he discovered that it was possible for colorimetric analysis to be performed in continually moving streams, thus eliminating the need for stepwise processing of samples and standards. The problem of deproteinisation, which 25 years ago was still a feature of most colorimetric methods of blood analysis, was solved by dialysis of the analyte from diluted blood across a membrane into a recipient stream early in the analytical pathway.

This "continuous flow" system, marketed by the Technicon Corporation as the "AutoAnalyser", began to be introduced into clinical laboratories in 1958 and remains to this day, through extensive modifications, the most widely used and successful one. Among the more important of later modifications have been the incorporation of additional physical measurements (flame photometry, fluorimetry, potentiometry with ion-specific electrodes) and the development of sequential multiple analysers where any number from 2 - 18 assays are performed in parallel on a single initial sample.[22]

The AutoAnalyser received an enthusiastic reception from influential clinical chemists in many parts of the world (its first public demonstration in England being in the prestigious environment of the Postgraduate Medical School in London[23]) and few misgivings were expressed on the revolutionary innovation it represented. One which was raised very early came from Tarnoky[24] who shrewdly observed in 1961:

> "The way in which a major piece of equipment influences thinking is of course not without its dangers and biochemists may come to think of new methods in terms of the automatic analyser they already have, even where a colorimetric method would not otherwise be the method of choice."

In 1964 Whitby,[25] after commenting on the extremely high cost of several Autoanalyser components, went on to observe:

> "... a danger of the complete adoption of automated techniques is that the staff required to operate machines may lose, or never acquire, the chemical knowledge and manual dexterity required for other laboratory work."

In other quarters the introduction of the Autoanalyser and the resulting boost in demands for this and other forms of instrumentation was greeted with delight! The following remarkably frank comment appeared in the issue of "Chemical and Engineering News" for December 1963:[26]

> "With bland indifference, many instrument companies a few years ago viewed the clinical chemistry laboratory as just another market. Some passed it off as a "hopelessly impoverished market" - chronically too short of funds to be really worth cultivating. Some regarded the demands of clinical chemistry laboratories as too special, too finicky to be worth fussing with.
>
> All that has changed. Dozens of instrument companies today are plunging into the clinical market with unprecedented zeal.

> Many believe that clinical laboratories and the broad bio-medical market have by far the greatest growth potential of any segment of the United States instrument business."

Since many features of the continuous flow system were protected by patent, frustrated instrument manufacturers turned their attention to automating the traditional methods of "test-tube" chemistry which had been bypassed by Skeggs when developing the AutoAnalyser. In place of sampling specimens and reagents by peristaltic action, mechanically driven syringes were employed, while reaction mixtures at various stages of analysis were held separate in individual tubes instead of as segments in a moving stream.

Two advantages were held by these "discrete" analysers compared with continuous flow systems.[27] Reaction mixtures for absorbance measurement could be presented to the colorimeter at a much faster rate - around 300 per hour compared with 60 per hour for most AutoAnalyser procedures. Secondly, smaller specimen volumes were often possible since the total final volume could be kept to the minimum required to be held stationary in the flow-cell, while losses in sensitivity due to incomplete dialysis were avoided.

The problem of deproteinisation, which could not easily be performed without interrupting the analysis for a centrifugation step, was largely solved by the development of many new and modified methods for the direct analysis of serum.[28]

The third type of automatic system, forming part of the "discrete" group but involving a radically new principle was

introduced by Anderson in 1969.[29] To measure the change in absorbance of reaction mixtures contained in a series of cuvettes in the shortest time, Anderson argued that the cuvettes, or the incident light beam, must move rapidly, one with respect to the other. He continued:

> "We have chosen to move the sample past the light beam by means of cuvette rotors. The centrifugal force inherent in this design has the advantage of providing the force for removing samples and reagents into the cuvette, for mixing them, for removing air bubbles, for sedimenting particulate matter and separating liquid phases."

With the exception of the centrifugal analyser, a comparative late-comer, automated systems had by the late 1960's been in clinical laboratories for around a decade and their impact was discussed in several reviews and symposia of that period. The general tone of these reviews can be gauged from short extracts from three of them:

> "... the wider application of automation will ensure that failure to provide adequate laboratory data, either at the level of fundamental discovery or for day-to-day patient care, will not restrict the development of biochemical knowledge or medical practice" (Moss, 1966).[30]

> "As a consequence (of automation) the overall laboratory efficiency is increased when this is measured in terms of the range of tests which can be offered, the quality of the results produced and the speed with which reports reach the clinician" (Nelson, 1969).[31]

> "There is no doubt that automatic methods of analysis are capable of making our lives easier; they reduce labour costs and increase speed. They are important companions in the laboratory today and will be increasingly so in the future" (Whitehead, 1969).[32]

Before moving on to discuss the reaction from the optimistic state evidenced above, as occurred during the 1970's, it is necessary to consider two consequences of automation, in respect of laboratory organisation, around which much controversy has arisen.

2.2 Organisational consequences for automation: Unsolicited multiple analyses

Screening procedures have been briefly mentioned earlier (Chapter 1) and regarded as a means to initiate the diagnostic process where clinical symptoms may be unobserved or absent. With regard to hospital patient admission screening, certain additional justifications were put forward, only some of which were concerned with the potential benefit of the patient.[33]

It was often probable that one or more of the tests comprising the screening battery might be asked for on a discretionary basis soon after admission, while others might be requested when the results of the earlier ones had been considered, or when involvement with the patient had passed up to more senior clinical staff. Provision of all these results at one time, very shortly after admission, would accelerate the diagnostic process, allow earlier introduction of treatment and shorten the patient's stay in hospital. It would also reduce to one the number of venepunctures

to which he was subjected.

In respect of the running costs of the laboratory service there were also advantages in the admission-screen approach. Of the sequence of events associated with the laboratory investigation of patients the actual analysis, performed automatically, had come to be the cheapest; the costs of transporting the specimen and preparing and returning the report were comparatively high. A single specimen and report could thus lead to substantial savings.

Finally, the performance of a whole battery of tests by means of multi-channel analysers - not only on the "admission" specimen but on nearly all specimens received - offered great convenience to the laboratory, for reasons which now follow:-

As shown earlier in Table I, the number of tests performed in SAIMR laboratories through the period 1952 - 1977 increased some twelvefold while the variety of tests little more than doubled. A relatively small number of different tests performed in large numbers make up a high percentage of the workload of present-day laboratories.[2]

Table III illustrates this in respect of the overall workload of all SAIMR laboratories for the nine month period January - December 1977. It will be seen that some 18 tests (about 10 per cent of the total variety performed) accounted for 90 per cent of the total workload, the latter being cut off at the level of the top 60 tests.

Consequently, the repertoire of just two high output multi-

TABLE III. Cumulative percentage frequencies for quantitative chemical tests in SAIMR laboratories, January - September 1977

ORDER OF FREQUENCY	TEST	NUMBER PERFORMED	CUMULATIVE FREQUENCY %
1	CHLORIDE (including CSF)	278 440	11,71
2	UREA	266 999	22,93
3	POTASSIUM	262 063	33,95
4	SODIUM	258 658	44,82
5	GLUCOSE (including CSF)	255 565	55,57
6	BICARBONATE (CO_2)	192 060	63,64
7	CREATININE	144 432	69,71
8	ASPARTATE TRANSAMINASE	60 033	72,24
9	BILIRUBIN	58 353	74,69
10	TOTAL PROTEIN	54 868	77,00
11	ALANINE TRANSAMINASE	53 044	79,23
12	ALKALINE PHOSPHATASE	49 762	81,32
13	URIC ACID	49 113	83,39
14	CHOLESTEROL	43 492	85,23
15	ALBUMIN	41 132	86,96
16	LACTATE DEHYDROGENASE	32 627	88,29
17	CALCIUM	28 761	89,50
18	PHOSPHORUS	23 627	90,49
19	CSF TOTAL PROTEIN	21 906	91,42
20	CREATINE KINASE	17 101	92,13
21	BLOOD pH AND GASES	15 883	92,80
22	PROTEIN ELECTROPHORESIS	14 193	93,40
23	TRIGLYCERIDES	12 434	93,92
24	THYROXINE	11 474	94,40
25	IRON/IRON BINDING CAPACITY	9 793	94,86
26	AMYLASE	9 688	95,27
27	URINE CREATININE	9 602	95,67
28	URINE POTASSIUM	6 178	95,93
29	URINE CHLORIDE	6 117	96,19
30	URINE UREA	6 060	96,44
	TOTAL	2 293 908	
	TOTAL FOR TOP 60 TESTS	2 378 570	(100%)

channel analysers - in this case the Technicon SMA 6/60 and 12/60 - could accommodate 90 per cent of the demands made on a laboratory.

Naturally, the repertoire of tests carried out on these high output analysers would be based on the frequency pattern of requests, but only rarely would the discretionary requests on any patient correspond exactly to the selection for which the analysers have been set up. Thus inevitably a large number of unsolicited assays would be carried out.

Until recently, however, this disadvantage was held to be well compensated by the streamlining of laboratory operations. Use of the multi-channel analyser, it was argued, avoided the necessity of allocating portions of the specimen to a number of different sites in the laboratory and later collecting and integrating separate items of result-data.

As summarised by Whitehead:[33]

> "A very high output analytical system is now available to us. The tests are based on the conventional discretionary-type analyses which have been part of clinical biochemistry for many years, but we are now moving into a situation where it is more convenient to perform biochemical profile tests than to limit requests to discretionary tests."

2.3 Organisational consequences of automation: The centralised "factory laboratory"

The move towards centralisation of clinical chemical laboratory work had its origin in the then unofficial system of referral of specialised tests (e.g. protein-bound iodine; urinary catecholamines)

to a small number of undertaking centres which had begun in the late 1950's.[34]

The advent of high output automation provided equipment which, initially at least, had a capacity in excess of the requirements for any single hospital patient population and proposals began to appear for the creation of central factory laboratories to process all the routine, automated work for a region comprising several hospitals.[35,36]

The advantages claimed for such an arrangement were discussed (together with the disadvantages) by the present author in 1971 (Appendix 1)[37] and are thus only outlined here. They include:

1. The more efficient use of costly equipment. As observed by Hargreaves[36] "it is not good economic sense for expensive equipment to be duplicated on sites within a few miles of one another while peripheral laboratories cannot get a simple colorimeter!"

2. Improvement in the uniformity and quality of the work, e.g. the use of the same reference value ranges and the existence of the same standards of precision and accuracy throughout the region.

A few isolated critics were gently chided by Nelson[31] when he wrote:

> "The idea of a factory laboratory to deal with a proportion of the routine workload appears to be a sensible solution to a current problem. I can see no reason why a clinical pathologist should feel any hurt to his professional pride by

> subcontracting the work in this way. He has been doing something quite like this for years by handing much of the work to laboratory technicians."

However, some chemical pathologists had begun to feel concern on grounds other than through affront to their professional pride, and the mounting tide of this concern must now be examined.

2.4 Counter-arguments to the introduction and utilisation of centralised high output automation

> "In a system in which quantity is equated with progress, where can one use a word like 'quality'?"

This final sentence of a letter appearing in the Lancet in January 1970[38] is symbolic of the change in attitude in the new decade, which gathered growing support as the decade progressed. The strength of this reaction even suggests that it might for some time have been lying dormant, waiting for someone to take the lead in its expression.

A number of reasons have been put forward[6] for the earlier reluctance to question the rising workloads:

1) The unpopularity attracted by a "reactionary" as opposed to a "progressive" attitude and approach.

2) The continuous effort (and further unpopularity) associated with efforts to control workloads contrasted with the relatively effortless expedient, for the pathologist and administrator, of installing automated equipment to process them.

3) The assumption, especially by non-clinically orientated laboratory workers, that all requests are necessary. The knowledge that utilisation of the laboratory had been steadily increasing for around forty years gave this phenomenon an appearance of inevitability in the cause of medical progress.

4) With workloads expanding everywhere else, failure to achieve this in one's own laboratory could be discreditable, whereas to expand even faster than the average trend would appear fashionable and impress colleagues. It could also prove a powerful argument to administrators for financing other intriguing novelties, such as electronic data-processing.

It will be convenient to consider the arguments against high output automation under a number of separate headings, some of which have been introduced earlier when discussing the potential benefits and other consequences of automation.

2.4.1 Costs

Central to the argument for the adoption of automation is that this cuts costs. While this is generally true as far as the cost per test is concerned, the total running costs of the laboratory will increase with the number of tests performed, many of which may be unnecessary. Zilva[39] has likened such a philosophy with taking a car on an unnecessary 200 mile motorway run solely for the purpose of cutting the cost per mile, while Griner and Liptzin commented:[40]

> "The ultimate impact of increased laboratory economy (on a cost per test basis) may be negligible, however, if physicians do not exercise restraint in ordering laboratory tests."

There are, furthermore, some "hidden extras" in the cost of operating automated laboratories. With workloads rising beyond the capacity of the formerly used manual methods to act as "back-up" it may be necessary to duplicate some of the equipment to provide for the eventuality of a breakdown. Alternatively, it will be necessary to negotiate service contracts with the equipment suppliers, providing for the use of loan equipment or rapid on-the-spot repair of faults, which can be equally costly.

2.4.2 Centralisation

Problems associated with the large-scale centralisation of routine biochemical work were considered by the author to fall into a number of groups (Appendix 1):[37]

a) Organisational problems associated with the delivery of specimens to and results from the laboratory.

b) The inflexibility resulting from "set" operating schedules for the automated equipment.

c) Problems in recruitment of staff to work under the factory-like conditions of the centralised laboratory.

2.4.3 Screening

Hospital admission screening has proved disappointing with regard to the number of clinically useful findings brought to light and disconcerting with regard to the number of abnormal unsolicited results which could not be explained.[41]

In the Birmingham study in 1967,[33] 2 071 patients were subjected to admission screening, generating 31 439 test results. This mammoth

exercise (equivalent to the year's total of tests at the SAIMR for 1946) resulted in a clinically useful finding in 172 patients (8,3 per cent), but in 748 patients (36,1 per cent) there was an unexpected abnormal result which could not be explained.

A similar pattern was found in a survey on 1 072 patients attending two group general practices in Edinburgh, carried out in the same year.[42] Of 15 110 analyses performed, 64 abnormal findings in 57 patients (5,5 per cent of the patients followed up) led to a change in the diagnosis or in the method of treatment, leaving a total of 259 unexplained abnormal results in 201 patients (19,3 per cent).

In summing up the results of multiphasic screening Galen[41] concluded that this, in general, is not effective, adding:

> "The laboratory tests used for health screening, because of their intrinsic nature and the low prevalence of disease are poorly predictive and therefore generate an unacceptable number of false positive findings."

2.4.4 The value of the laboratory

Various of the above detrimental features of high output automation and the factory laboratory have been stressed by different writers. Nearly all these criticisms, however, contain as a common feature concern over the declining usefulness of the laboratory in medical care, when considered in relation to the escalating costs of laboratory services.

The actual range of tests commonly carried out has been critically examined.[43] With the exception of some cellular enzymes

(e.g. aspartate and alanine transaminases) and, occasionally, tests such as serum iron, the "admission profile" comprises the same range of 12 - 20 tests which have been available for the past fifty years and which, according to Marks,[43] "owe their popularity at least as much to feasibility, availability, familiarity and habit as to clinical utility except in a few well-defined diseases".

Percy-Robb and co-workers[42] expressed a similar view when they suggested that "... the ease with which (high output analysers) undertake their task distracts attention from fundamental questions such as whether they are performing the most appropriate range of investigations and performing them reliably".

There are many other causes for the distraction of the chemical pathologist from such fundamental questions. Some of the non-medical preoccupations of the modern laboratory head were described in the earlier quotation from Potgieter,[14] while Powell[44] as early as 1970 had commented:

> "This rapid increase in the volume of work tends to make us, as pathologists, more concerned about the standard deviation of a batch of results in a quality control programme than with the elucidation of a single abnormality in a given patient. It takes less time for the pathologist to suggest a further list of tests than it would to see the patient for himself."

The dwindling amount of time available to the pathologist to perform a consultative function is, ultimately, the most serious adverse consequence of the spiralling workload. As stated at the

same early period by Zilva:[39]

> "Good relations between clinical and laboratory staff and an exchange of ideas and knowledge (either in the selection or the interpretation of the tests) is in the best interests of the patient. This ideal is not encouraged by pressure on the laboratory to carry out tests indiscriminately."

The pressures on chemical pathologists have been further aggravated by serious shortages in their own ranks.[45] As remarked by Carter and co-workers[6] "while production of results in itself may be satisfying to some, recruitment, especially of good medical personnel, to centralised laboratories, often geographically remote from the patients they serve, has already become difficult".

A possible avenue of escape from the unhappy situation outlined in the foregoing pages was suggested by the author[37] in 1971 and is expressed in the following quotation, which serves as a summary of this chapter:-

> "The continuous increase in the workload of hospital clinical chemistry departments has given rise to several problems in the last 20 years.
>
> The initial problem, the increase in analytical work, has in large measure been solved by the introduction of mechanisation. Subsequently, the problem of dealing with the greatly increased amount of paper-work is in the process of being solved by the development of computer-based data-processing systems.
>
> However, the factory-like conditions of the present-day laboratory, with deterioration or

severance of relations between the laboratory and the hospital now presents a set of problems to which no ready solution is apparent.

It is suggested that the growth of any single laboratory should not be allowed to proceed beyond an optimum level which allows enjoyment of the benefits of modern technology but avoids the detrimental features of overloading. To this end, instead of centralisation we should be moving towards the expansion of existing small laboratories and the creation of new laboratories.

Until such time as the growing workload of these laboratories justifies the introduction of full automation, use can be made of work-simplified systems or 'limited function automation'."

CHAPTER 3

3. WORK SIMPLIFICATION AND LIMITED FUNCTION AUTOMATION IN THE CLINICAL CHEMISTRY LABORATORY

3.1 Introduction

Developments in analytical equipment and methods in all fields of chemistry during the past sixty years have frequently had as their objective a reduction in the work of the analyst, either as manually performed operations or as visual observations.

As early as 1932 Peters and Van Slyke[15] drew attention to the then newly developed "automatic" balances which obviated the use of the smaller weights and the method of "weighing by swings", thus greatly expediting gravimetric determinations. In another example from the same authors the reagents for the tungstic acid deproteinisation of blood (sodium tungstate, sulphuric acid and water) which, in the earlier Folin-Wu procedure[46] were added to the blood in succession, were all mixed in advance so that only one pipetting was required.

Instrumental developments, such as those of the photoelectric colorimeter or the flame photometer were epoch-making advances in work simplification. Finally, many of the modifications in the basic chemistry of clinical chemical methods were characterised at the time of publication as being "simplified" methods.

However, the term "work simplification" appears to have been first deliberately used and discussed at a Symposium entitled "Semiautomatic Analytical Systems" held by the Association of Clinical Biochemists in Birmingham in April 1966.[47,48]

In introducing the term Mitchell, Profitt and Annan[47] gave it a fairly wide meaning, considering the Autoanalyser itself to represent an advanced form of work simplification. Seligson,[48] defining simplification as "reduction in complexity", included under this heading the elimination of unnecessary administrative and analytical steps and the incorporation of labour-saving procedures and devices whenever possible.

In a broad sense, work simplification can be applied to all those aspects of laboratory work which might be investigated by a work-study consultant, including the physical lay-out of the working bench, the proximity and accessibility of other equipment, the sequence of operations, both clerical and technical, and so forth.

The term "limited function automation", introduced by Hardy in the early 1960's and more fully described by him in 1968,[21] had a more restricted application, being initially confined to the semiautomation of fluid measurement by means of hand-controlled mechanically driven samplers, dispensers and diluters.

In this dissertation the term limited function automation is employed in a sense equivalent to the terms "semiautomation" or "partial automation" used by other workers[49] and covers two main classes of application:

1) The use of mechanical and computational aids to supplement or replace manually or mentally performed operations at all appropriate stages of the analysis. Such aids will generally be employed in the second group of operations discussed in Chapter 2 (skilled operations involving measurement or

calculation) and include automatic pipettes, automatic diluters, automatic aspiration of solution into the measuring cuvette, timing of photometric measurements, calculation of concentration or activity and printing out of results.

2) The use of special instruments, usually dedicated to the estimation of only one or two analytes, which carry out many of the above operations in an integrated sequence (as in full automation) but which require manual presentation of the sample and sometimes operator-involvement at other stages.

The use of special analysers of this type (IL 343 Flame Photometer; Radiometer chloride meter; Harleco microgasometer) will be described in the next chapter when methods for particular analyses are discussed. The more general-purpose items of equipment for limited function automation will be described in the remaining part of this chapter.

3.2 Equipment for limited function automation

A large number of different semiautomatic liquid-handling systems and flow-through calculating absorptiometers are available commercially. Rather than attempt to review all the models available, attention will be focussed on those which were used for the experimental portion of this dissertation and which have been widely introduced into SAIMR laboratories.

3.2.1 Liquid-handling systems: General considerations

Depending on the mechanism used for the measurement and movement of fluid, a basic division into two types of device can be made:

1) Peristaltic devices. These employ plastic or rubber tubing compressed between forward moving rollers, as introduced by Skeggs[12] for fluid monitoring on the AutoAnalyser. This approach is employed in some dispenser/diluters (e.g. the General Diagnostics Compu-Pet) and also in the autodiluter module of the IL 343 Flame Photometer.

2) Syringe-action devices. These can be further divided into those where the syringe plunger is operated manually and those where it is driven mechanically, usually by an electric motor.

The dispensers and diluter used in this work all fell into the manually operated, syringe action category.

3.2.2 Oxford Sampler Micropipetting Systems (Figure 1)

These are available with various capacities, delivering fixed volumes ranging from 5 microlitres to 1 millilitre; multi-range samplers combining two or three volumes have also been developed. The spring-loaded syringe barrel is moved by a push-button operated by the thumb, displacing air from the syringe via a plastic tip fitted at the delivery end. On releasing the pressure liquid can be drawn up into the tip and later displaced by re-pressing the button to a greater extent to expel all the liquid.

3.2.3 Oxford Macro-Set Transfer Pipetting system (Figure 2)

This is similar in appearance to the Oxford Sampler but has a larger capacity and is capable of variable adjustment to deliver volumes in the range 1 - 5 ml.



Figure 1. Oxford Sampler Micropipetting Systems.



Figure 2. Oxford Macro-Set Transfer Pipetting System.

3.2.4 Clay-Adams Selectapette (Figure 3)

This pipette is constructed in the shape of a "gun" with a hand grip; the delivery tip is set at an angle to the syringe and is capable of being extended with a tip-adaptor to allow access to the bottom of a 15 cm test tube. The Selectapette has a delivery capacity of 1,0 ml, adjustable in steps of 0,1 ml.

3.2.5 Scientific Manufacturing Industries SMI Micro/Pettor (Figure 4)

In this pipette the syringe barrel consists of a precision bore capillary made of glass (1 - 200 µl volumes) or plastic (200 - 1 000 µl volumes). Liquid to be dispensed is drawn up and expelled by stainless steel plunger, usually fitted with a Teflon tip.

The SMI Kit 1075-C provides for a range of volumes from 10 - 30 µl in steps of 5 µl, while the Kit 1075-I can dispense volumes of 50, 60, 75, 80 and 100 µl.

3.2.6 Oxford Pipettors (Figure 5, left)

Essentially, these comprise a hand-operated syringe mechanism mounted on top of a bottle which serves as a reservoir of the liquid to be dispensed. The syringe is filled from the reservoir by a tube dipping below the surface of the liquid and its contents expelled via a delivery jet on a side arm.

Two models of Oxford Pipettor were used in this work, the Model R with dispensing range 0,1 - 1,0 ml and the Model SA with dispensing range 1,0 - 10,0 ml.



Figure 3. Clay-Adams Selectapette.



Figure 4. SMI Micro/Pettor.



Figure 5. Oxford Model SA Pipettor (left) and Model 20 Dilutor (right).

3.2.7 Oxford Model 20 Dilutor (Figure 5, right)

Automatic diluters commonly employ two syringes, a smaller one (5 - 100 µl) to take up the sample and a larger one (500 µl - 10,0 ml) for the diluent.

In the case of the Oxford Dilutor the sample volume, fixed at 20 µl, is taken up into the elongated tip of the side arm itself and then washed out with the diluent volume, adjustable from 1,0 - 10,0 ml, by operating the main syringe in the same manner as with the Model SA Pipettor.

3.2.8 Gilford Microsample Spectrophotometer, Model 300-T (Figure 6)

The Gilford 300-T, seen on the right hand side in Figure 6, comprises a tungsten light source, diffraction grating monochromator, linear absorbance photometer, four-place light-emitting diode display, rapid-sampling system and a temperature-controlled cuvette.

The light source and monochromator provide a spectral range of 300 - 740 nm; the wavelength setting disc is calibrated from 312 - 729 nm. In practical terms the spectral range extends down low enough to permit kinetic enzyme and other determinations at 340 nm.

The rapid-sampling system is operated from a suction pump via a glass vacuum reservoir. The volume of liquid aspirated into the flow-cell, at a constant suction pressure generally set at 10 pounds/square inch, is regulated by a timing mechanism which holds open the connecting valve for periods adjustable from 0,1 - 1,0 second in steps of 0,1 second. The minimum recommended

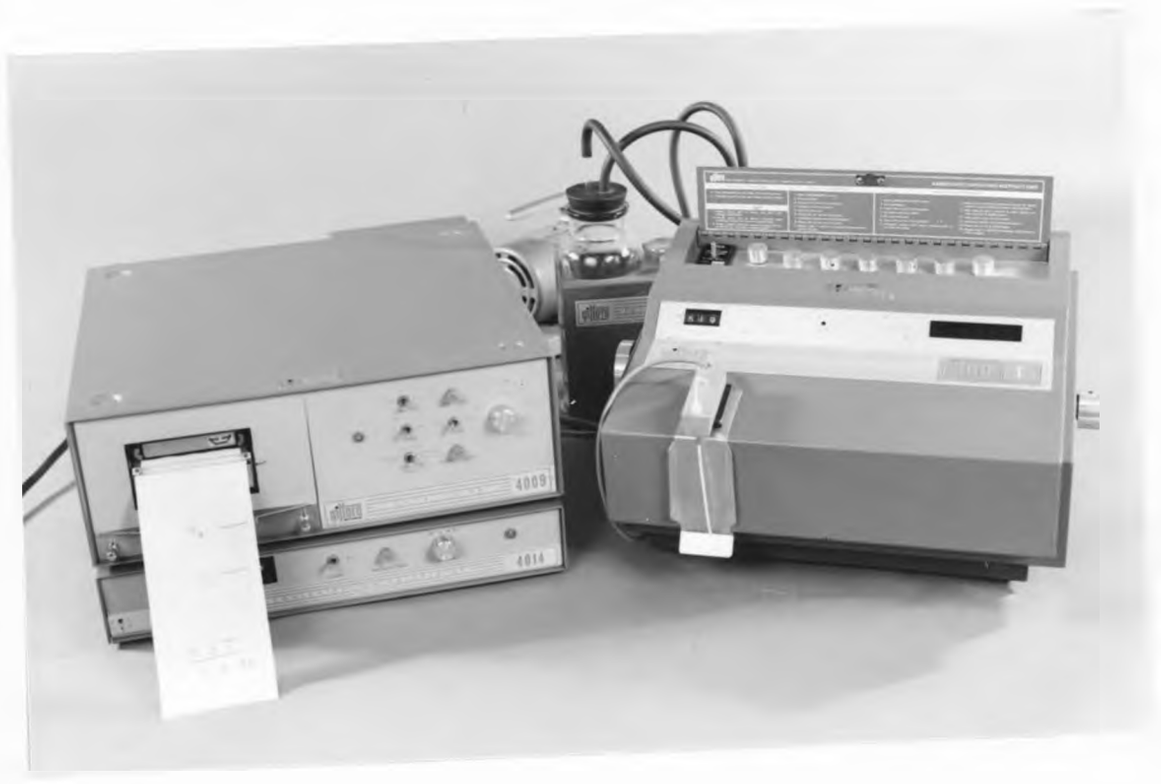

Figure 6. Gilford Microsample Spectrophotometer and Data Lister.

sample volume is 500 µl.

The temperature control for the cuvette can be set at 25°C, 30°C, 32°C or 37°C; display of the actual working temperature can be obtained at the LED read-out. Photometric readings can be displayed either as absorbance or in units of concentration; in the latter case, after setting to zero on the reagent blank and sampling a standard of known concentration, the display is set to this concentration value by means of a calibration control.

Absorbance and concentration data from the spectrophotometer can be transmitted to the Gilford 4009 Data Lister, shown on the left hand side of Figure 6. The print-out provided can either be single-time values, as each of a succession of samples are aspirated, or can be a series of readings at ten second intervals which follow the change in absorbance of a single solution held in the flow cell. This latter facility is used in kinetic determinations or for monitoring the course of the reaction when developing absorptiometric methods.

Finally, the rate of change in absorbance in such serial measurements can be converted to activity or concentration units by means of the Gilford 4014 Enzyme Calculator. Although available, this Gilford accessory was not considered essential for supply to SAIMR Branch Laboratories and was not employed in the experimental work described later.

3.3 The merits of limited function automation

Instruments for automated analysis are needed in clinical chemistry to reduce manual work, increase the productivity of laboratory staff,

improve the quality of results and, thereby, enhance the contribution of clinical chemistry to the improvement of patient care.[19]

The early introduction of the continuous-flow system resulted in many larger laboratories making an immediate transition from conventionally performed manual methods to full automation, but brought no benefit to the many more smaller, less affluent laboratories. Subsequently, the disparity between the two types of laboratory became even more marked; at the time when the Technicon SMA 6/60 was introduced into the central laboratories of the SAIMR (1972) many peripheral laboratories were still measuring urea by the urease/Nessler method and bicarbonate on the volumetric Van Slyke apparatus.

The principal value of limited function automation is to extend to such smaller laboratories facilities, appropriate to their workload, allowing them to fulfil the objectives stated in the opening paragraph of this section.

In introducing the concept, Hardy[21] numbered amongst its advantages the facts that it is relatively inexpensive, requires no special skill or training for its operation and can readily be adapted to conventional manual procedures already familiar in the laboratory. For the smaller hospital laboratory limited function automation offers an attractive alternative to the remote factory laboratory, permitting the continued local performance of much of the local workload.

After describing the respective features of the different systems of full automation, Mitchell[50] wrote in 1969:

"... it must be remembered that for various reasons certain aspects of our work will not be suitable for complete automation for many years, if ever. For these there is the simple form of automation termed 'work simplification', development of which has been proceeding in parallel with that on the more complex machines.

There are those amongst us who maintain that simplifying as much as possible is more practical than installing complex machines, and they prove their point in their own laboratories most effectively."

CHAPTER 4

4. SELECTION OF ASSAYS AND ANALYTICAL METHODS FOR STUDY

4.1 Selection of assays

A primary practical objective of this work was related to the responsibilities of the Biochemical Methods and Equipment Unit at the SAIMR. This Unit was established in 1976 with the aim of standardising analytical methods, equipment and quality control procedures throughout the Regional Laboratory Service operated by the Institute.

Although the Central Institute was founded in 1914, it was not until 1930 that the first Branch Laboratory, at Port Elizabeth, was set up. By 1949 the number of Branches had only risen to three, but subsequent expansion of the Regional Laboratory Service, particularly during the past 15 years, led by the end of 1979 to the existence of 60 laboratories, distributed as shown in Figure 7.

Only a minority of these laboratories have workloads justifying the introduction of full automation, at the end of 1979 there being seven Technicon SMA 6/60 analysers and six Abbott ABA-100 discrete analysers in use in the entire SAIMR Laboratory Service. The remaining laboratories were thus appropriate sites for the introduction of limited function automation.

The survey on overall frequencies of chemical tests (Table III) had given an indication of the direction this study should take. It was felt necessary, however, to confirm these findings in respect of a cross-section of individual laboratories.

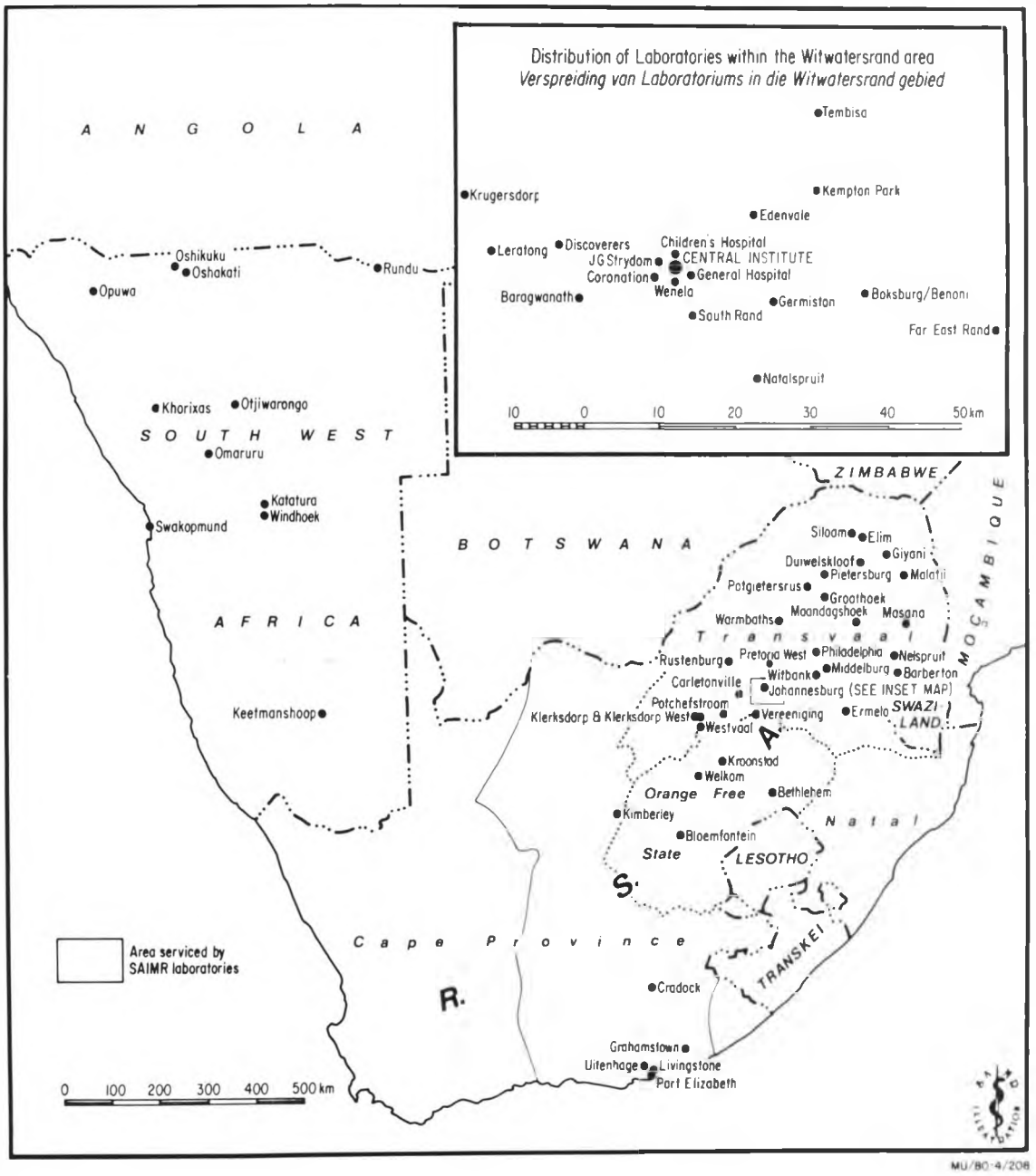

Figure 7. Branch Laboratories of the South African Institute for Medical Research, December 1979.

Workloads, confined to the top 18 quantitative blood analyses, were therefore tabulated for the following six Branch Laboratories for the year 1977:-

Hospital	Population group served	Number of beds
Natalspruit	Black	870
Coronation	Coloured/Asian	585
Childrens' Hospital	White	240
Germiston	White	262
Krugersdorp	White	267
Pietersburg	White Non-White	255 396 } 651

The above laboratories were selected on the basis of diversity of patient population and included one (Pietersburg) situated outside the Witwatersrand area in the predominantly rural Northern Transvaal. In order that the workloads would not be influenced by the production pattern of multiple analysers such as the SMA 6/60, none of the laboratories had any form of full automation apart from Coronation, where a home-made five-channel system of Autoanalyser equipment was in use for electrolyte and urea determinations.

Comparison of this data (Table IV) with the overall pattern given in Table III shows a general similarity, with electrolytes, urea and glucose high on the list in all cases. There are, however, some noticeable differences in the work patterns:

a) Due to the low incidence of requests, cholesterol determinations were not performed at all at the two entirely non-white hospitals (Natalspruit and Coronation), specimens

TABLE IV. Biochemical test workloads at six SAIMR Branch Laboratories, 1977

	NATALSPRUIT		CORONATION		CHILDRENS' HOSPITAL		GERMISTON		KRUGERSDORP		PIETERSBURG	
Work units												
Biochemistry:												
Total	408 963 (48,8%)		585 609 (39,1%)		348 989 (36%)		177 728 (35,9%)		179 319 (35,8%)		183 240 (30,8%)	
Per bed	470		1 001		1 454		678		672		281	
Bacteriology	210 229		459 410		300 354		151 004		190 440		256 829	
Haematology	219 210		452 612		319 637		166 736		131 513		155 124	
TOTAL	838 402		1 497 631		968 980		495 468		501 272		595 193	
Tests												
1	Potassium	14 570	Potassium	26 697	Potassium	7 504	Urea	3 605	Urea	2 739	Urea	2 866
2	Urea	14 546	Urea	26 415	Urea	6 915	Potassium	3 512	Potassium	2 574	Chloride	2 542
3	Sodium	14 353	Sodium	25 882	Sodium	6 893	Sodium	3 433	Sodium	2 473	Potassium	2 355
4	Chloride	14 308	Chloride	25 698	Chloride	6 722	Chloride	3 420	Chloride	2 466	Sodium	2 263
5	CO_2	13 970	CO_2	15 723	Glucose	2 820	CO_2	3 414	CO_2	2 384	CO_2	1 955
6	Glucose	4 107	Glucose	9 885	CO_2	2 045	Uric acid	1 719	Glucose	1 521	Cholesterol	1 837
7	ALP	1 489	Creatinine	6 759	Creatinine	1 594	Glucose	1 622	Uric acid	1 331	Uric acid	1 786
8	Total protein	1 431	AST	2 842	Calcium	1 526	AST	479	AST	1 108	Glucose	1 367
9	Creatinine	1 407	LD	2 787	Albumin	1 365	ALT	453	ALT	1 082	ALP	1 146
10	AST	1 343	ALT	2 746	Total protein	1 203	LD	417	CK	985	Bilirubin	1 027
11	Bilirubin	1 334	Albumin	2 543	AST	795	Cholesterol	405	LD	794	AST	924
12	ALT	1 314	Total protein	2 491	ALT	782	CK	377	Bilirubin	721	Albumin	846
13	LD	1 298	Bilirubin	1 876	Bilirubin	771	Bilirubin	358	ALP	689	Total protein	711
14	Albumin	1 207	Uric acid	1 659	ALP	728	Creatinine	249	Total protein	613	ALT	665
15	Amylase	1 096	ALP	1 235	LD	644	ALP	229	Albumin	597	Triglycerides	549
16	Calcium	646	Calcium	1 171	Phosphorus	634	Calcium	179	Cholesterol	419	LD	296
17	Phosphorus	493	CK	1 147	Uric acid	473	Albumin	176	Calcium	144	CK	278
18	Uric acid	294	Amylase	1 121	Iron	274	Total protein	173	Creatinine	131	Calcium	187

for this test being sent to the Central Institute.

b) In a number of cases creatinine is displaced downwards in order of popularity (at Pietersburg coming 19th). This suggests either that the relative technical difficulty of the manual analysis inhibits its ready performance and availability, or that the contribution of the seven SMA 6/60s, all performing creatinine on one of the channels, boosted this assay in the overall figures.

c) The proportion of biochemical work in the total workload is generally around 30 - 40 per cent, being highest in the two non-white hospitals.

d) The intensity of patient investigation (reflected by the work-units per bed) is highest in the urban teaching hospitals (Coronation and Childrens' Hospital) and lowest in the rural hospital (Pietersburg).

The eventual selection of assays for study comprised the following:-

a) The group of seven tests heading the list in Table III, i.e. electrolytes, urea, glucose and creatinine.
These analyses would be likely to be in the immediate repertoire of a newly-opening Branch Laboratory and include the four tests (sodium, potassium, chloride and bicarbonate) requiring special instrumentation for limited function automation.

b) One representative from each of the following groups:-

1) Protein group - Albumin.
2) Mineral group - Calcium.
3) Lipid group - Cholesterol

This choice was influenced by the need to investigate alternatives to the methods routinely in use in several of the Branches for these three assays (salt-fractionation for albumin, complexiметric titration for calcium, Liebermann-Burchard reaction for cholesterol).

For other assays which might have been considered on the basis of popularity the existing methodology was more satisfactory (diazo reaction for bilirubin, biuret reaction for total protein, phosphomolybdic acid reduction for phosphorus, phosphotungstic acid reduction for uric acid).

c) Following an initial trial of the methods selected for the above assays in the Wellcome Quality Control Programme (Section 4 of this chapter), it was decided to extend the number of assays from ten to twelve by the addition of two enzyme determinations, alkaline phosphatase and aspartate transaminase.

4.2 Methods and equipment

The bases for decision-making in respect of analytical methods and equipment were:

a) Existing procedures in the Branch Laboratories as surveyed at the end of 1976.

b) Comparative performance figures for different techniques provided in the six-monthly end-of-cycle surveys of the Wellcome Quality Control Programme.[51]

A compilation of data for the most frequently used methods for two cycles of the Programme between April 1977 and March 1978

is presented in Table V. In this Table concentrations are expressed in the "traditional" units then in use by SAIMR participants (which are satisfactory for comparative purposes); precision figures denote the between-batch repeatability of randomly duplicated samples while "bias" figures relate the mean value for all twelve samples in the cycle to the overall mean for all participants. For the two enzyme assays, only numbers of users of the different methods are given, since methodological differences in numerical results make inter-comparison difficult.

Prior to about 1970 the majority of SAIMR laboratories were equipped with the EEL-100 Flame Photometer for sodium and potassium assays and the volumetric Van Slyke[52] apparatus for total CO_2 determinations. Chloride was measured by the Schales[53] mercuric nitrate titration procedure, while most of the remaining assays involved colorimetric methods read on the Leitz filter colorimeter.

Re-equipping of the Branches with more modern apparatus began early in the 1970's, and evaluations carried out in that period led to the standardised introduction into selected laboratories of a number of items, including:

IL 343 Flame Photometer,

Radiometer CMT-10 Chloride Titrator,

Harleco Micro CO_2 Analyser,

Beckman Glucose Analyser,

Beckman BUN Analyser.

Later, this standardisation was extended to include the Gilford

TABLE V. Performance of various procedures in the Wellcome Quality Control Programme, 1977-78

Procedure	Cycle 1			Cycle 2		
	No.	Precision	Bias	No.	Precision	Bias
Sodium (mmol/l)						
IL 343, 543, 743	126	1,7	-0,044	147	1,7	-0,048
IL 143 or 243	103	1,8	-0,37	103	1,8	-0,52
Corning 450 or 430	60	1,7	-0,37	83	1,9	-0,28
EEL 100 or 150	43	3,7	0,54	48	3,6	-0,063
Total manual	447					
Total	880					

SAIMR 1976

EEL 100	18
IL 143	1
Beckman Klina	1
IL 343 or 543	15

TABLE V (continued)

Procedure	Cycle 1			Cycle 2		
	No.	Precision	Bias	No.	Precision	Bias
Potassium (mmol/l)						
IL 343, 543, 743	126	0,093	-0,006	148	0,098	-0,004
IL 143 or 243	106	0,088	Zero	103	0,130	0,001
Corning 450 or 430	61	0,097	0,014	84	0,110	0,019
EEL 100 or 150	44	0,170	-0,013	51	0,160	-0,046
Total manual	452					
Total	885					

SAIMR 1976

EEL 100	18
IL 143	1
Beckman Klina	1
IL 343 or 543	15

TABLE V (continued)

Procedure	Cycle 1			Cycle 2		
	No.	Precision	Bias	No.	Precision	Bias
Chloride (mmol/l)						
Schales titration	141	3,1	1,1	134	3,0	1,2
Corning 920 chloride meter	103	2,0	-1,0	128	1,9	-1,0
Marius chloride meter	43	1,8	-0,79	47	2,1	-0,85
Radiometer chloride meter	32	1,9	-0,48	43	1,8	-0,58
IL 279, colorimetric	20	1,9	0,36	25	2,1	0,056
Beckman chloride/CO_2 analyser	-	-	-	19	3,0	0,069
Total manual	416			463		
Total	762			847		

SAIMR 1976

Schales titration	26
Radiometer chloride meter	9

TABLE V (continued)

Procedure	Cycle 1			Cycle 2		
	No.	Precision	Bias	No.	Precision	Bias
Bicarbonate (mmol/l)						
Harleco Micro CO_2 Analyser	120	1,7	-0,55	130	1,9	-0,27
Blood gas analyser	21	3,6	2,0	25	3,2	1,8
Beckman chloride/CO_2 analyser	-	-	-	20	1,4	-2,0
Van Slyke titration	16	2,2	3,6	18	1,9	3,7
Natalson Gasometer	14	2,0	-0,73	15	1,9	-0,26
Volumetric Van Slyke	5	1,5	-1,2	5	1,9	-1,5
Total manual	223			244		
Total	586			641		

SAIMR 1976

Harleco	20
Volumetric Van Slyke	12

TABLE V (continued)

Procedure	Cycle 1			Cycle 2		
	No	Precision	Bias	No.	Precision	Bias
Urea (mg/100 ml)						
Urease/Berthelot	159	6,9	-1,6	164	6,0	-1,8
Diacetyl monoxime	40	6,0	-1,5	41	6,0	-1,3
Beckman BUN analyser	14	8,7	-5,0	25	9,2	-2,5
Urease/GLDH	19	9,3	-4,3	17	6,2	-1,6
Urease/Nessler	3	4,6	2,8	5	5,9	0,90
Total manual	237			268		
Total	901			1 006		

SAIMR 1976

Urease/Nessler	15
Beckman BUN analyser	14
Urease/Berthelot	5

TABLE V (continued)

Procedure	Cycle 1			Cycle 2		
	No.	Precision	Bias	No.	Precision	Bias
Creatinine (mg/100 ml)						
Jaffé with deproteinisation	120	0,24	0,006	122	0,24	0,016
Jaffé: Corrected for non-specific chromogen	34	0,18	0,030	32	0,18	0,039
Jaffé: Kinetic	28	0,35	-0,044	38	0,31	-0,14
Jaffé: With Lloyds reagent	21	0,19	-0,032	21	0,21	-0,038
Total manual	215			234		
Total	823			914		

SAIMR 1976

Jaffé with deproteinization	17
Jaffé: Corrected for non-specific chromogen (Slot modification)	2

TABLE V (continued)

Procedure	Cycle 1			Cycle 2		
	No.	Precision	Bias	No.	Precision	Bias
Glucose (mg/100 ml)						
Beckman analyser	85	5,3	-4,4	97	5,5	-3,7
Glucose oxidase/PERID	51	7,7	-7,1	52	5,9	-6,3
Hexokinase/G6PD (UV)	34	7,7	5,6	45	10,0	3,8
Glucose oxidase/PAP without deproteinisation	34	6,7	0,10	38	6,4	-0,76
Glucose oxidase/PAP with deproteinisation	30	6,3	-3,7	36	6,7	-2,2
Hexokinase/G6PD (Glucostrate)	27	6,1	2,7	28	8,1	3,7
O-Toluidine (without deproteinisation)	25	9,0	-1,6	32	6,2	-1,5
Total manual	338			382		
Total	907			1 009		

SAIMR 1976

Copper reduction (colorimetric)	19
Beckman analyser	14
Hexokinase/G6PD (Glucostrate)	2

TABLE V (continued)

Procedure	Cycle 1			Cycle 2		
	No.	Precision	Bias	No.	Precision	Bias
Albumin (g/100 ml)						
Cellulose acetate electrophoresis, Ponceau-S stain	115	0,27	-0,45	131	0,24	-0,40
BCG Dye Binding: Citrate buffer	75	0,23	0,094	82	0,23	0,092
BCG Dye Binding: Lactate buffer	53	0,26	0,074	54	0,24	0,060
BCG Dye Binding: Succinate buffer	49	0,20	0,130	50	0,21	0,14
Salt fractionation	4	0,26	-0,67	10	0,27	-0,56
Total manual	326			364		
Total	720			807		

SAIMR 1976

BCG Dye Binding (lactate buffer)	12
Salt fractionation - biuret	10
BCG Dye Binding (other buffers)	5
Cellulose acetate electrophoresis	1

TABLE V (continued)

Procedure	Cycle 1			Cycle 2		
	No.	Precision	Bias	No.	Precision	Bias
Calcium (mg/100 ml)						
Methylthymol blue	68	0,48	0,069	80	0,44	0,10
EGTA/Corning-EEL 940	53	0,27	-0,23	72	0,24	-0,20
Varian atomic absorption	35	0,39	0,022	36	0,38	0,018
Cresolphthalein colorimetric	32	0,53	0,12	38	0,52	0,063
EDTA/Calcein	21	0,40	-0,032	20	0,59	-0,16
Total manual	385			418		
Total	821			919		

SAIMR 1976

EDTA/Calcein	14
Glyoxal-Bis colorimetric	4
Cresolphthalein colorimetric	3

TABLE V (continued)

Procedure	Cycle 1			Cycle 2		
	No.	Precision	Bias	No.	Precision	Bias
Cholesterol (mg/100 ml)						
Liebermann-Burchard without extraction	93	11	15	109	12	17
Cholesterol oxidase/catalase	75	11	-10	77	10	-10
Cholesterol oxidase/PAP	28	10	-11	53	9,8	- 9,6
Fe^{+++} salt/H_2SO_4	36	14	5,5	34	13	4,2
Dimethylbenzene sulphonic acid/H_2SO_4	19	12	15	20	11	12
Total manual	290			331		
Total	775			885		

SAIMR 1976

Liebermann-Burchard without extraction	18
Ferric chloride/sulphuric acid	4
Dimethylbenzene sulphonic acid/acetic anhydride	4
Cholesterol oxidase/catalase	1
Cholesterol oxidase/phenol/4-aminophenazone	1

TABLE V (continued)

Procedure	Cycle 1	Cycle 2
	No.	No.
Alkaline phosphatase		
Phenolphthalein phosphate	71	75
PNPP: Diethanolamine	50	53
Kind and King	37	38
PNPP: AMP	19	34
PNPP: Eskalab	24	25
King-Armstrong	9	12
Total manual	241	278
Total	850	958

SAIMR 1976

Phenolphthalein phosphate	11
King-Armstrong	10
Kind and King	7

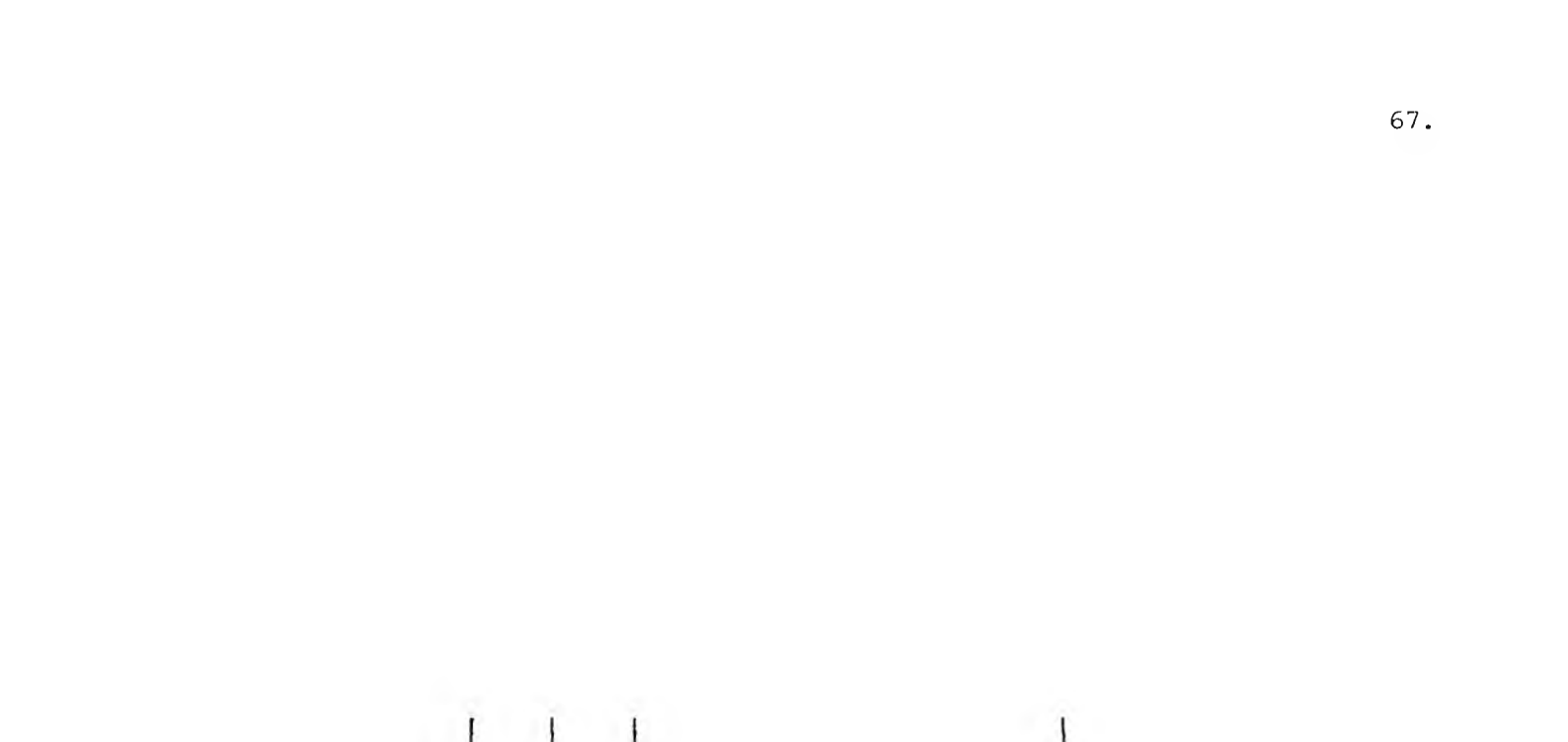

TABLE V (continued)

Procedure	Cycle 1	Cycle 2
	No.	No.
Aspartate transaminase		
Rate-Reaction (UV): All aspartate concentrations	157	179
Reitman-Frankel and modifications	39	41
"Transac": Fast violet B	31	34
Total manual	228	255
Total	835	943

SAIMR 1976

Reitman-Frankel and modifications	13
"Transac" (Fast violet B)	7
Rate-Reaction (UV)	1



300-T spectrophotometer, twelve of which were issued to the Branches early in 1977.

Examination of the Wellcome end-of-cycle surveys indicates that selection of the IL Flame Photometer and the Harleco CO_2 Analyser conforms to the world-wide popularity of these two instruments. Regarding the Radiometer chloride meter our choice appears to be more individualistic, but performance figures for this model compare satisfactorily with those of the more popular chloride meters.

Although several Beckman BUN and Glucose Analysers had been installed in SAIMR laboratories by 1976, it was more recently decided to discontinue the introduction of further instruments for the following reasons:-

1) As shown in Table V, the BUN Analyser has a precision inferior to that of even most manual methods for urea estimation.

2) Use of the Glucose Analyser for additional polarographic determinations (e.g. uric acid) proved inconvenient and was hardly ever carried out in the Branch Laboratories.

3) It was not economically feasible to supply one or both of these analysers as well as the Gilford and therefore a choice had to be made. The Gilford, while particularly required for kinetic enzyme determinations at 340 nm, was a general-purpose instrument and could be used in the limited function automation of manual glucose and urea methods.

4.3 Analytical techniques

4.3.1 Presentation

Descriptions of the methods used for the twelve assays are presented in the following general format:-

a) Analytical principle. Since the methods used are all well-established and documented only an outline of the principle is provided in most instances.

Part of the work on the validation of the methods, described later, involved correlation of results on patients' specimens with those obtained with the Technicon SMA 6/60 and 12/60. In addition to the manual methods, therefore, the principles of the automated methods are also given.

b) Reagents and standards. Extensive use was made in this work of commercially supplied analytical kits and made-up reagents, since this practice can significantly reduce the manual working time (and possibility of error) associated with the laboratory-based preparation of reagents. As observed by Mitchell,[2] the use of pre-packaged reagents has shown the value of the high level quality control which is possible in the commercial mass production of this material - a degree of control difficult to attain in the frequently understaffed smaller laboratory.

Where commercial reagents were employed an outline of their overall composition, either as supplied by the manufacturer or as given in the original literature reference, is included.

c) Working instructions for the analyses. For simplicity in presentation these are given, where appropriate, as in a

practical manual, using the present active rather than the past passive form. Where a standard was set up calculation of results was made in the usual way by the expression:

$$\frac{\text{reading of test}}{\text{reading of standard}} \times \text{concentration of standard.}$$

For the fairly small numbers of tests performed at any one time use of the above formula, aided by a pocket calculator, was found to be as convenient as setting up the direct concentration print-out of the Gilford. Observing the actual absorbance readings of standards and blanks was, furthermore, useful in day-to-day quality assurance.

4.3.2 Sodium and potassium

Principle. Both the manual and SMA 6/60 methods are based on lithium internal reference flame photometry, introduced by Berry, Chappell and Barnes[54] in 1946.

Manual determinations were carried out on the IL 343 Flame Photometer (Figure 8) using reagents supplied by the manufacturer. The automatic diluter at the bottom centre of the instrument takes up the sample, stock lithium nitrate solution (3 000 mmol/l) and water to give a 1/200 dilution of the sample at a lithium concentration of 15 mmol/l. This solution is then atomised and fed into the air/propane flame.

The sample requirement is around 0,1 ml for the two simultaneous determinations and the analysis time is approximately 20 seconds.

Standardisation is effected with the same serum-based



Figure 8. IL 343 Flame Photometer.

standard (General Diagnostics Automated Lock-In) used for the SMA 6/60, having as typical values 141 mmol/l for sodium and 5,0 mmol/l for potassium.

4.3.3 Chloride

Manual determinations were carried out on the Radiometer CMT-10 Chloride Titrator (Figure 9) using reagents supplied by the manufacturer.

Principle. The operating principle is that of coulometric titration, introduced by Cotlove[55] in 1958.

When the chloride-containing solution for analysis is added to the electrolyte solution (0,4 mol/l H_2SO_4) contained in the plastic titration cup, a chloride-sensing silver/silver chloride electrode causes a power generator and timing counter to switch on.

The power generator applies a controlled voltage between a pair of silver electrodes and the resulting current liberates silver ions from the anode at the bottom of the cup. These immediately react with chloride ions, forming a precipitate of insoluble silver chloride which is stabilised in suspension by gelatine present in the supporting electrolyte.

When the chloride ion concentration is reduced to a very low level, at the end-point of the titration, the chloride-sensing electrode causes the power generator and timing circuit to switch off. As the current intensity and the amount of delivered sample are known, the time for complete titration is a measure of the chloride concentration in the sample, which is displayed directly in millimols per litre.



Figure 9. Radiometer CMT-10 Chloride Titrator.

SMA 6/60. The automated method for chloride determination is a colorimetric one,[56,57] based on the replacement of thiocyanate by chloride in an acidified mercuric thiocyanate solution. Subsequent reaction with ferric ions forms red coloured ferric thiocyanate.

Procedure. Sample volumes in the range 1,0 - 30 µl can be analysed on the Radiometer Titrator; in this work the volume used was 20 µl, measured with an SMI Micro/Pettor. Standardisation was effected with an aqueous sodium chloride solution containing 100 mmol/l, prepared from Merck "Titrisol" concentrated volumetric solution. The process of standardisation involves adjusting the "pipette volume" thumbwheels to a reading where analysis of the standard gives the correct value on two successive occasions.

Depending on the chloride concentration being measured the analysis time for serum is around 25 - 30 seconds.

4.3.4 Bicarbonate

Manual assays were carried out with the Harleco Micro CO_2 Analyser (Figure 10) using reagents supplied by the manufacturer.

Principle. The Harleco method is a simplified version of that of Van Slyke,[52] involving volumetric measurement of the CO_2 liberated from serum or plasma by acidification and subsequent agitation.

SMA 12/60. The automated method for bicarbonate is a colorimetric one[57] in which the CO_2 liberated by dilute sulphuric acid diffuses across a silicone rubber membrane into a weak alkaline buffer containing cresol red as a pH indicator. The



Figure 10. Harleco Micro CO_2 Analyser.

increase in absorbance of this stream at 420 nm is directly proportional to the CO_2 content of the sample.

Reagents. CO_2 liberating solution: 250 ml of 85 per cent lactic acid made up to one litre with water.

Bicarbonate standard (25 mmol/l): 2,100 g sodium bicarbonate made up to one litre with water.

Gas capillary indicating fluid: 0,1 g Azure A, 1,0 g benzoic acid and 0,5 ml Tween 20 made up to one litre with water.

Procedure. Pipette 500 µl of the liberating reagent into the right-hand section of the plastic reaction cup and 100 µl of serum on standard into the left-hand section, using Oxford Samplers for both measurements.

Lower the gasometer head on to the cup and tighten the gasket against the top of the cup by means of the screw until the upper meniscus of the blue indicating fluid is close to the zero mark on the scale.

Some drift in the zero setting was found to occur during the next few seconds and for the best precision the manufacturer's instructions were modified by making the final adjustment to zero after a pause of 15 seconds.

Switch on the automatic shaker to agitate the assembly and cause the acid reagent to mix with the sample. When agitation ceases, after 30 seconds, adjust the capillary and scale to the upright position and immediately read off the gas volume to the nearest 0,5 scale division.

4.3.5 Urea

In selecting a manual urea method the choice lay between the urease/Berthelot procedure[58] and a direct diacetyl monoxime method. In earlier work (Georges, 1973[59]) the latter had been found to be well adaptable to limited function automation, employing simple reagents of good long term stability. Some comparative studies using the urease/Berthelot method are presented in Chapter 5.

In this work, application of the direct method was further simplified by using reagents identical to those used on the SMA 6/60.[60,61]

Principle. In a relatively weak acid solution diacetyl monoxime is hydrolyzed to diacetyl which in turn reacts directly with urea[62] in the presence of ferric ions. The presence of thiosemicarbazide intensifies the yellow colour of the reaction product.[63]

Reagents. Reagents as formulated by Technicon for the SMA 6/60 were obtained commercially (CSL) and had the following composition:-

Colour reagent. 1,675 g diacetyl monoxime, 0,335 g thiosemicarbazide and 0,3 g Brij 35 made up to one litre with water.

Acid reagent. 33 mg ferric chloride hexahydrate and 0,67 ml 85 per cent phosphoric acid made up to one litre with 20% (V/V) sulphuric acid.

Standard. An aqueous solution of urea containing 10,0 mmol/l.

Procedure. This is essentially the method described by Wootton,[64] but uses 10 µl of sample in place of 100 µl of a 1/10 dilution.

Pipette into a series of 15 x 1,5 cm test tubes:

2,0 ml water (Oxford Pipettor),

10 µl serum or standard (SMI) (no addition is made to the blank),

2,0 ml colour reagent (Oxford Pipettor): Vortex mix,

2,0 ml acid reagent (Oxford Pipettor): Vortex mix thoroughly.

Place the tubes in a boiling water bath for 20 minutes.

Cool and read the pink coloured solutions on the Gilford at 520 nm.

4.3.6 Creatinine

Principle. Both the manual and automated procedures are based on the colour reaction of creatinine with alkaline picrate solution, first described by Jaffé[65] in 1886.

The manual method developed for use is a scaled-down version of that of Brod and Sirota,[66] simplified by the use of a combined alkaline picrate reagent in place of separate additions of picric acid and sodium hydroxide to the protein-free filtrate. Use of a combined reagent had earlier been investigated in relation to urine creatinine estimation (Georges, 1976[67]). Whereas other formulations[68] of this reagent require its preparation immediately before use, the formulation adopted allowed for use of the reagent after standing up to one week at room temperature, although with some rise in the reagent blank absorbance reading.

Reagents. Saturated picric acid solution (approximately 13 g/l) (CSL).

1 mol/l sodium hydroxide, prepared from Merck "Titrisol" concentrated volumetric solution,

50 g/l sodium tungstate (CSL),

0,33 mol/l sulphuric acid (CSL),

Alkaline picrate reagent: Equal volumes of the picric acid and sodium hydroxide solutions mixed together.

Standard. An aqueous creatinine solution containing 200 µmol/l, prepared by dilution of a stock solution (10 mmol/l) comprising 0,113 g creatinine made up to 100 ml with 0,1 mol/l hydrochloric acid.

Procedure. Pipette into a series of 10 x 1 cm test tubes:

0,5 ml serum or standard (Oxford Sampler),

0,5 ml water (1,0 ml for the blank) (Selectapette),

0,5 ml sodium tungstate solution (Selectapette),

0,5 ml sulphuric acid, with shaking (Selectapette).

Vortex mix, stand 10 minutes and centrifuge:

1,0 ml protein-free filtrate (Selectapette),

0,5 ml alkaline picrate (Selectapette).

Mix and stand 15 minutes out of direct illumination, then read on the Gilford at 520 nm.

4.3.7 Glucose

In selecting a manual glucose method the choice lay between two

enzymatic procedures, using either glucose oxidase or hexokinase for the primary reaction. In the Wellcome surveys hexokinase methods showed in general an inferior precision to those based on glucose oxidase, although the 1977 CAP survey[69] showed little difference between the two. Earlier work by the author[70] in 1971 had found that a colorimetric kit based on the hexokinase reaction (General Diagnostics "Glucostrate") had poor precision, especially at high levels (CV 5,9% at a level of 16,5 mmol/l) and, at that time, suffered virtually quantitative interference from fructose. Consequently, it was decided to adopt the glucose oxidase method developed by Trinder,[73] using the reagent kit supplied by the Boehringer Mannheim Corporation.

Principle. The aldehyde group of glucose is oxidised by glucose oxidase to produce gluconic acid and hydrogen peroxide. The latter, through the action of peroxidase, oxidises phenol in the presence of 4-aminophenazone to give the pink coloured product 4 (p-benzoquinone mono-imino) phenazone which is measured colorimetrically at 500 nm.

SMA 12/60. The automated procedure[72] is based on the reduction by glucose of cupric neocuproine (2:9 dimethyl 1:10 phenanthroline) to an intense orange yellow cuprous complex, after earlier addition of sodium carbonate to the sample stream to reduce interference by ascorbic acid and sulphydryl groups.

Reagents. The working reagent of the Boehringer kit has the following composition:-

0,1 mol/l phosphate buffer, pH 7,4; 4-aminophenazone 0,77 mmol/l; phenol 11 mmol/l; glucose oxidase $\geq$ 18 U/ml.

peroxidase ≥ 1,1 U/ml.

Standard. An aqueous glucose solution containing 10 mmol/l, made up in benzoic acid solution (10 g/l).

Procedure. The method developed for use contains two modifications from the Boehringer kit instructions. In place of 200 µl of a 1/10 protein-free filtrate, the reaction is carried out directly on 20 µl of plasma (blood being collected in tubes containing 3 mg potassium oxalate and 1 mg sodium fluoride for each millilitre of blood to act as anticoagulant and preservative). Secondly, colour development is carried out for 15 minutes at 37°C instead of for 15 - 20 minutes at 25°C.

Pipette into a series of 10 x 1 cm test tubes:

2,0 ml working reagent (Oxford macrosampler),

20 µl sample or standard (SMI) (no addition is made to the blank).

Briefly vortex mix, then incubate at 37°C for 15 minutes.

Read on the Gilford at 500 nm.

4.3.8 Albumin

Since only few SAIMR laboratories were performing cellulose acetate electrophoresis this method, although evidently popular among participants in the Wellcome Programme, was deemed to be impracticable. Of the various methods based on dye-binding with bromocresol green the General Diagnostics "Albustrate" method, using a lactate buffer at pH 4,0 was selected on the basis of its commercial availability and existing use in a number of the Branches. The same product, prepared to the formula given

by Babson,[73] subsequently became available locally from Clinical Science Laboratories.

A further convenience is the fact that the same reagent is used in the SAIMR for albumin determination of the SMA 12/60 and other continuous-flow analysers.

Principle. The estimation of albumin by means of bromocresol green is based on the affinity of albumin at a pH of around 4,0 for the blue coloured anionic form of the indicator, the resulting complex having an absorption peak at 630 nm.

Reagent. Lactate buffered BCG was obtained commercially from CSL and had the following composition:-

Lactic acid/sodium hydroxide buffer, 0,1 mol/l, pH 4,0; bromocresol green 100 mg/l; Tween-20 2 ml/l. The stock reagent is diluted 1/5 with water to give the working reagent used in the assay.

Standard. The standard used was the same serum-based one (Technicon SMA Calibrating Serum) used with the SMA 12/60, having as a typical value 46 g/l of albumin.

Procedure. Pipette into a series of 10 cm x 12 mm test tubes:

1 ml stock BCG reagent (Selectapette),

4 ml water (Oxford Pipettor).

Mix, then add:

10 µl sample or standard (SMI) (no addition is made to the blank).

Mix by inverting several times after covering the mouth of the tube with parafilm (vortex mixing was found not

to give a good dispersion of the serum into the reagent). Stand for 10 minutes, then read on the Gilford at 630 nm.

4.3.9 Calcium

The widespread use of the colour reaction of calcium with cresolphthalein complexone[74] in continuous-flow analysis (including the SMA 12/60) suggested that this approach should be used also for the manual procedure. The method adopted was the microprocedure of Baginski,[75] using reagents obtained commercially from CSL.

A possible reason for the relative unpopularity of this colour reagent for manually performed analyses compared to the use of methylthymol blue[76] (as indicated in the Wellcome surveys) is the more noticeable interference from calcium contamination found with CPC as a result of its high sensitivity. In continuous-flow analysis, where the manifold is continually cleansed by the flow of reagents, this problem is less serious. To avoid such contamination the two reagents were dispensed from an Oxford Dilutor and Pipettor respectively, while colour development was carried out in small plastic tubes. Furthermore, reagent blank solution was aspirated several times through the flow-cell of the Gilford prior to photometric measurements, in order to wash out calcium. These simple precautions reduced the between-batch standard deviation on the Wellcome Programme to less than half the overall mean value for this method.

Principle. Serum is treated directly with a CPC reagent

containing 8-hydroxy quinoline which eliminates interference from magnesium.[77] After alkalinisation the purple colour is measured at 575 nm.

Reagents. Colour reagent (CSL): 50 mg cresolphthalein complexone, 3,125 g 8-hydroxyquinoline and 100 ml dimethyl sulphoxide made up to 1 litre with water.

Base reagent (CSL): 156 ml of 2-amino 2-methyl 1-propanol made up to one litre with water.

Standard (CSL): An aqueous calcium standard containing 2,50 mmol/l.

Procedure. Using the Oxford Dilutor, sample 20 µl of serum or standard and wash into a 4 ml capacity plastic tube with 1 ml of colour reagent (contained in the reservoir of the Dilutor). The blank consists of 1 ml of colour reagent only. Add 1 ml of the base reagent from an Oxford Model R Pipettor. Vortex mix and read on the Gilford at 575 nm.

4.3.10 Cholesterol

As indicated in the Wellcome surveys, the fully enzymatic method of Allain *et al.*[78] as exemplified by the Boehringer cholesterol kit (CHOD/PAP procedure), has rapidly gained in popularity and, by virtue of its simplicity and reliability, is now the method of choice. The same procedure and reagents are employed on the SMA 12/60 at the SAIMR. Some comparative studies using a modified Liebermann-Burchard procedure[79] are presented in Chapter 5.

Principle. Cholesterol esters are hydrolyzed by cholesterol ester hydrolase. The free cholesterol is then oxidised by

cholesterol oxidase to yield cholest-4-en-3-one and hydrogen peroxide. The latter, in the presence of peroxidase, then reacts with 4-aminophenazone and phenol in the same manner as in the glucose procedure described earlier.

Reagents. The working reagent of the Boehringer kit has the following composition:-

Phosphate buffer 0,4 mol/l, pH 7,7; phenol 10 mmol/l; 4-aminophenazone 1 mmol/l; methanol 1,85 mol/l; cholesterol ester hydrolase 200 U/ml; cholesterol oxidase 60 U/ml; peroxidase 40 U/ml; hydroxy poly-ethoxy dodecane 2 g/l.

Standard. (Boehringer "Preciset"): 5,18 mmol/l (200 mg/100 ml) cholesterol made up in iso-propanol.

Procedure. Pipette into a series of 10 x 1 cm test tubes:

2,0 ml working reagent (Oxford macrosampler),

20 µl sample or standard (SMI) (no addition is made to the reagent blank).

Briefly vortex mix, then incubate at 37°C for 15 minutes. Read on the Gilford at 500 nm.

4.3.11 Alkaline phosphatase (orthophosphoric-monoester phosphohydrolase, E.C. 3.1.3.1)

The method introduced by Babson[80] in 1966, based on the use of phenolphthalein monophosphate as substrate and commercially available in kit form (General Diagnostics "Phosphastrate Alkaline") shows the highest individual popularity in the Wellcome surveys. By 1976 this method had already overtaken the long-established King-Armstrong[81] procedure in popularity in SAIMR laboratories.

In an appraisal of the method Wilkinson and Vodden[82] commented in 1966:

> "The method for determination of serum alkaline phosphatase using phenolphthalein monophosphate as substrate can be recommended for further evaluation, since it is simple, reliable and sensitive, and may readily be adapted for automation."

Georges and Persona[83] described in 1979 a scaled-down version of the Phosphastrate kit method in which all volumes are reduced to one-fifth of the original. As further economies the working substrate was prepared by dilution of the General Diagnostics preparation supplied in bulk for automated analysis, while calibration was effected with a phenolphthalein standard curve (as originally described by Babson) instead of through the use of commercial calibrating sera.

Principle. At a pH of 10,15 alkaline phosphatase splits phosphate from phenolphthalein monophosphate, producing from the free phenolphthalein a pink colour which is intensified and stabilised by addition of a phosphate buffer at pH 11,2.

SMA 6/60. The automated method determines alkaline phosphatase by a procedure based on that of Bessey, Lowry and Brock.[84] The enzyme acts on a substrate of p-nitrophenol phosphate at a pH of 10,25 to liberate p-nitrophenol with a yellow colour which, after dialysis, is measured in the flow-cell at 410 nm.

Reagents, procedure and standardisation. These are described in the paper by Georges and Persona[83] (Appendix 2). The substrate

obtained from General Diagnostics comprises 65 mmol/l phenolphthalein monophosphate dicyclohexylamine monohydrate in 7,8 mol/l 2-amino-2-methyl-1-propanol buffer at pH 10,15.

4.3.12 Aspartate transaminase (L-Aspartate: 2-oxoglutarate aminotransferase, E.C. 2,6.1.1)

As indicated in the Wellcome surveys, reaction-rate methods are steadily replacing two-point colorimetric methods for this determination, being in use in over 70 per cent of laboratories by 1978. The non-availability of measuring devices suitable for reading at 340 nm with temperature control of the cuvette prevented the use of reaction-rate methods in most SAIMR branches before 1977, when Gilford spectrophotometers began to be introduced.

Principle. The assay employs the coupled reaction introduced by Karmen[85] in 1955. Oxalacetate formed from 2-oxoglutarate and aspartate by the primary transamination reaction is reduced by malate dehydrogenase in the presence of NADH to form malate and NAD. This second reaction is monitored by following the decrease in absorbance at 340 nm.

SMA 12/60. The same Karmen procedure is used in the automated method, but with a single-point measurement of the decrease in absorbance at 430 nm.[86]

Reagents. Assays were carried out using reagents supplied by Worthington Diagnostics ("Statzyme GOT"). The working substrate has the following composition:-

0,05 mol/l phosphate buffer, pH 7,4; L-aspartate 133 mmol/l; 2-oxoglutarate 6,6 mmol/l; NADH 0,23 mmol/l; lactate dehydrogenase $\geq$ 2 000 U/l, malate dehydrogenase $\geq$ 100 U/l.

Lactate dehydrogenase is added to remove serum pyruvate rapidly during the first minute (lag phase) of the reaction; pyruvate could otherwise act as a substrate for serum lactate dehydrogenase, in the presence of NADH, causing an additional fall in absorbance at 340 nm.

Procedure. The method adopted differed from that given in the Worthington instruction sheet in respect of the volumes of substrate and serum used. The latter procedure employs 0,2 ml serum with 3,0 ml substrate; under these conditions the enzyme activity is calculated from the change in absorbance per minute by means of a factor derived as follows:-

$$f = \frac{3,2}{6,22} \times \frac{10^3}{0,2} = 2\ 572$$

where

3,2 = total reaction volume,

0,2 = serum volume,

6,22 = absorbance of NADH at a concentration of 1 µmol/ml,

10^3 = number of millilitres in one litre.

Multiplying the change in absorbance per minute by this factor gives the enzyme activity in units per litre.

In the method adopted volumes were scaled down and a higher proportion of serum to substrate was employed, leading to a smaller factor. With the microvolume flow-cell of the Gilford it is

possible to have as reaction mixture 0,7 ml substrate and 0,1 ml serum; under these conditions the above factor becomes

$$\frac{0,8}{6,22} \times \frac{10^3}{0,1} = 1\ 286$$

The smaller factor reduces the influence of instrumental "drift" on the final result. As the reference range for healthy persons is approximately 6 - 26 U/l, a non-specific change in absorbance of 0,001 absorbance units will correspond to an activity of around 2,6 U/l with the original Worthington procedure (i.e. 10 per cent of the upper limit). The smaller factor reduces this possible source of error by 50 per cent.

Procedure. Using the Selectapette dispense 0,7 ml of the substrate into a series of small test tubes and place these in a water bath at 30°C adjacent to the Gilford. Set the temperature control on the Gilford to 30°C and the wavelength to 340 nm. Sample a portion of the substrate into the flow cell and back off the absorbance reading to zero.

For each test, add 100 µl serum with an SMI Micro/Pettor to a tube of substrate, briefly vortex mix and sample into the Gilford. Switch on the timer-printer and monitor the reaction over three minutes. Calculate the mean change in absorbance over the second and third minutes.

4.4 Preliminary assessment of the selected methods in the Wellcome Quality Control Programme

Nine of the initial selection of ten assay-methods were tested during a cycle of the Wellcome Programme between 10/10/77 and 13/3/78. A Harleco Micro CO_2 Analyser was available on loan at

a later date for the precision and reference range studies (Chapters 5 and 6) but was not available for the five month period covered by the Programme.

Procedure. Specimens consisted of a set of twelve lyophilised control sera, reconstituted by addition of 10,0 ml water. A single "A" grade volumetric pipette was used throughout for this purpose. All the assays were carried out singly, using the methods described in the previous section.

Data-processing of the submitted results by Wellcome Reagents Limited in London provided the following information for each assay on each fortnightly specimen:-

a) The overall mean, standard deviation and number of results.

b) The mean, standard deviation and number of results for our specific method.

c) The bias of our result, relative to the method mean, expressed as the number of standard deviations "plus" or "minus".

In addition, the "end of cycle" report gives information on the between-batch precision of various specimens which were duplicated during the cycle. In order to "disguise" some of these duplicated samples, the analyte levels are slightly varied by dispensing different volumes of the same bulk serum prior to lyophilisation.

In this cycle the related specimens and the corresponding volume correction factors were as follows:-

	Related samples	Multiplication factor
Pool A	Specimens 1 and 8	1,000
Pool B	Specimens 2, 6 and 12	1,102
	5	0,913
	9	1,003
Pool C	Specimens 3 and 10	1,000
Pool D	Specimens 4 and 11	1,050
	7	0,954

In the following Table (Table VI, a - j) the fortnightly results are presented in sequence and the values for related samples, duly corrected, are subsequently shown grouped together.

Calculation of the between-batch precision was performed by the Programme Organisers from the following equation:-

$$SD = \sqrt{\frac{\Sigma_A^D (x_1 - \bar{x})^2}{\Sigma_A^D (N - 1)}}$$

where

x_1 = each individual result,

$\bar{x}$ = mean result for the set of replicates,

N = number of replicates of each pool.

The precision data relating to all methods, to our method and to our individual results are presented in Table VII.

4.4.1 Discussion

a) Accuracy. With the exception of one glucose result (specimen 2)

TABLE VI. Performance of nine selected methods in the Wellcome Quality Control Programme

(a) SODIUM (mmol/l)

QC No.	All methods Mean	Method Mean (x)	SD	Result (y)	Bias (SD)
1	129,5	129,5	2,0	131	0,7
2	127,0	127,0	2,3	127	Zero
3	145,4	145,2	2,3	146	0,3
4	132,6	132,6	2,1	134	0,7
5	153,2	152,7	3,0	153	0,1
6	127,1	127,0	2,2	127	Zero
7	145,9	145,6	2,3	145	-0,3
8	129,6	129,6	2,2	131	0,6
9	139,7	139,5	2,2	140	0,3
10	145,3	145,2	2,3	146	0,3
11	132,7	132,7	2,2	130	-1,2
12	127,2	127,0	2,2	126	-0,5
Mean	136,27	136,13		136,33	0,20 mmol/l (0,15%)
SD	9,17	9,05		9,23	

RELATED VALUES	A	B	C	D
	1) 131	2) 140	3) 146	4) 140,7
	8) 131	6) 140	10) 146	11) 136,5
		12) 138,9		7) 138,3
		5) 139,7		
		9) 140,4		

Regression equation: $y = 1{,}011\,x - 1{,}29$ mmol/l

$r = 0{,}992$

TABLE VI continued

(b) POTASSIUM (mmol/l)

QC No.	All methods Mean	Method Mean (x)	SD	Result (y)	Bias (SD)
1	4,44	4,44	0,11	4,5	0,5
2	3,33	3,33	0,11	3,3	-0,3
3	5,42	5,40	0,13	5,4	Zero
4	4,16	4,16	0,12	4,2	0,4
5	4,04	4,03	0,10	4,1	0,7
6	3,33	3,33	0,11	3,3	-0,3
7	4,59	4,59	0,11	4,6	0,1
8	4,45	4,44	0,10	4,5	0,6
9	3,67	3,67	0,11	3,7	0,2
10	5,41	5,40	0,15	5,4	Zero
11	4,16	4,16	0,11	4,1	-0,5
12	3,34	3,34	0,11	3,3	-0,3
Mean	4,195	4,191		4,200	0,009 mmol/l (0,2%)
SD	0,725	0,721		0,734	

RELATED VALUES	A		B		C		D	
	1)	4,5	2)	3,64	3)	5,4	4)	4,41
	8)	4,5	6)	3,64	10)	5,4	11)	4,31
			12)	3,64			7)	4.39
			5)	3,74				
			9)	3,71				

Regression equation: $y = 1,016\ x - 0,059$ mmol/l

$r = 0,998$

TABLE VI continued

(c) CHLORIDE (mmol/l)

QC No.	All methods Mean	Method Mean (x)	SD	Result (y)	Bias (SD)
1	90,9	90,0	2,2	90	Zero
2	87,1	86,2	2,2	86	-0,1
3	106,3	105,8	2,2	108	1,0
4	91,1	90,3	2,4	90	-0,1
5	104,9	104,6	2,5	106	0,6
6	87,0	86,1	2,1	86	Zero
7	100,2	99,6	2,0	101	0,7
8	91,0	90,2	2,5	91	0,3
9	95,9	95,2	2,4	99	1,6
10	106,2	105,9	2,4	108	0,9
11	91,0	90,3	2,2	88	-1,0
12	86,9	86,2	1,9	86	-0,1
Mean	94,88	94,20		94,92	0,72 mmol/l (0,76%
SD	7,60	7,79		8,89	

RELATED VALUES

A		B		C		D	
1)	90	2)	94,77	3)	108	4)	94,50
8)	91	6)	94,77	10)	108	11)	92,40
		12)	94,77			7)	96,35
		5)	96,78				
		9)	99,30				

Regression equation: $y = 1{,}130\ x - 11{,}56$ mmol/l

$r = 0{,}991$

TABLE VI continued

(d) UREA (mmol/l)

QC No.	All methods Mean	Method Mean (x)	SD	Result (y)	Bias (SD)
1	20,58	19,56	1,50	20,83	0,8
2	9,92	9,98	1,12	9,33	-0,6
3	14,93	14,88	1,58	16,33	0,9
4	10,83	10,72	1,11	11,09	0,3
5	12,00	11,96	0,86	11,85	-0,1
6	9,91	9,87	0,89	9,26	-0,7
7	11,93	11,79	0,66	11,50	-0,4
8	20,52	20,19	2,06	20,55	0,2
9	10,93	11,10	1,10	11,07	Zero
10	14,83	14,54	1,49	15,11	0,4
11	10,77	10,50	0,95	11,10	0,6
12	9,87	9,90	0,91	9,87	Zero
Mean	13,085	12,916		13,158	0,242 mmol/l (1,9%)
SD	3,884	3,652		4,105	

RELATED VALUES

A		B		C		D	
1)	20,83	2)	10,28	3)	16,33	4)	11,64
8)	20,55	6)	10,20	10)	15,11	11)	11,66
		12)	10,88			7)	10,97
		5)	10,55				
		9)	11,10				

Regression equation: $y = 1,115\ x - 1,25$ mmol/l

$r = 0,992$

TABLE VI continued

(e) CREATININE (µmol/l)

QC No.	All methods Mean	Method Mean (x)	SD	Result (y)	Bias (SD)
1	429,6	416,4	35,4	397,8	-0,5
2	187,4	190,1	17,7	185,6	-0,3
3	265,2	267,0	23,9	265,2	-0,1
4	208,4	209,9	20,8	217,0	0,3
5	226,1	229,4	22,7	212,2	-0,8
6	188,0	191,8	17,8	186,9	-0,3
7	229,3	233,8	21,4	225,7	-0,4
8	430,8	418,5	40,9	401,1	-0,4
9	206,5	208,7	21,1	214,7	0,3
10	265,1	267,2	23,6	255,3	10,5
11	209,0	210,6	21,1	209,1	-0,1
12	188,5	192,6	20,3	186,9	-0,3
Mean	252,83	253,00		246,46	-6,54 µmol/l (2,6%)
SD	86,95	81,12		75,63	

RELATED VALUES

A	B	C	D
1) 397,8	2) 204,5	3) 265,2	4) 227,9
8) 401,1	6) 206,0	10) 255,3	11) 219,6
	12) 206,0		
	5) 193,7		
	9) 215,3		

Regression equation: $y = 0,929\ x + 11,40$ µmol/l

$r = 0,996$

TABLE VI continued

(f) GLUCOSE (mmol/l)

QC No.	All methods Mean	Method Mean (x)	SD	Result (y)	Bias (SD)
1	3,64	3,54	0,36	3,44	-0,3
2	4,75	4,77	0,43	5,67	2,1*
3	8,43	8,08	0,67	8,50	0,6
4	7,25	7,11	0,61	6,94	-0,3
5	5,72	5,80	0,49	5,74	-0,1
6	4,71	4,70	0,36	4,61	-0,2
7	7,96	7,83	0,43	7,54	-0,7
8	3,62	3,54	0,36	3,47	-0,2
9	5,16	5,10	0,40	5,11	Zero
10	8,42	8,27	0,52	8,19	-0,2
11	7,21	7,03	0,36	6,96	-0,2
12	4,69	4,68	0,40	4,56	-0,3
Mean	5,963	5,871		5,894	0,023 mmol/l (0,4%)
SD	1,798	1,727		1,733	

RELATED VALUES	A	B		C		D	
1)	3,44	2)	[6,25]	3)	8,50	4)	7,29
8)	3,47	6)	5,08	10)	8,19	11)	7,31
		12)	5,03			7)	7,19
		5)	5,24				
		9)	5,13				

Regression equation: $y = 0,986\ x + 0,11$ mmol/l

$r = 0,983$

TABLE VI continued

(g) ALBUMIN (g/l)

QC No.	All methods Mean	Method Mean (x)	SD	Result (y)	Bias (SD)
1	31,4	31,4	2,9	32	0,2
2	38,9	40,4	3,0	41	0,2
3	34,2	34,6	2,8	36	0,5
4	32,9	33,8	2,8	34,5	0,3
5	46,0	47,7	4,2	50	0,5
6	38,9	40,1	3,2	42	0,6
7	36,0	36,6	2,8	39	0,9
8	31,5	31,5	2,4	32	0,2
9	42,4	43,7	3,7	45	0,3
10	34,1	34,6	2,8	36	0,4
11	32,8	33,9	2,8	35	0,4
12	38,8	40,3	3,2	41	0,3
Mean	36,49	37,38		38,63	1,25 g/l (3,34%)
SD	4,58	5,07		5,43	

RELATED VALUES

A		B		C		D	
1)	32,0	2)	45,18	3)	36,0	4)	36,23
8)	32,0	6)	46,28	10)	36,0	11)	36,75
		12)	45,18			7)	37,21
		5)	45,65				
		9)	45,14				

Regression equation: $y = 1{,}065\ x - 1{,}19$ g/l

$r = 0{,}994$

TABLE VI continued

(h) CALCIUM (mmol/l)

QC No.	All methods Mean	Method Mean (x)	SD	Result (y)	Bias (SD)
1	2,523	2,523	0,128	2,55	0,2
2	2,230	2,280	0,128	2,15	-1,0
3	3,023	2,973	0,183	3,05	0,4
4	2,188	2,213	0,105	2,20	-0,1
5	2,691	2,706	0,143	2,76	0,4
6	2,227	2,270	0,109	2,26	-0,1
7	2,414	2,432	0,097	2,31	-1,3
8	2,520	2,530	0,101	2,50	-0,3
9	2,447	2,480	0,118	2,50	0,2
10	3,026	2,980	0,137	3,01	0,2
11	2,190	2,223	0,101	2,21	-0,1
12	2,233	2,298	0,134	2,27	-0,2
Mean	2,476	2,492		2,481	-0,011 mmol/l (0,44%)
SD	0,302	0,270		0,312	

RELATED VALUES

A		B		C		D	
1)	2,55	2)	2,37	3)	3,05	4)	2,31
8)	2,50	6)	2,49	10)	3,01	11)	2,32
		12)	2,50			7)	2,20
		5)	2,52				
		9)	2,51				

Regression equation: $y = 1{,}140\ x - 0{,}360$ mmol/l

$r = 0{,}987$

TABLE VI continued

(j) CHOLESTEROL (mmol/1)

QC No.	All methods Mean	Method Mean (x)	SD	Result (y)	Bias (SD)
1	3,32	3,00	0,33	2,95	-0,2
2	3,23	2,99	0,29	2,85	-0,5
3	3,40	3,17	0,32	3,16	Zero
4	2,82	2,60	0,30	2,49	-0,4
5	3,88	3,58	0,42	3,50	-0,2
6	3,23	2,94	0,31	2,93	Zero
7	3,08	2,84	0,28	2,73	-0,4
8	3,31	2,98	0,26	2,98	Zero
9	3,51	3,26	0,39	3,19	-0,2
10	3,46	3,19	0,37	3,18	Zero
11	2,77	2,54	0,31	2,37	-0,5
12	3,18	2,98	0,36	2,87	-0,3
Mean	3,266	3,006		2,933	-0,073 mmol/1 (2,4%)
SD	0,299	0,281		0,312	

RELATED VALUES

A		B		C		D	
1)	2,95	2)	3,14	3)	3,16	4)	2,62
8)	2,98	6)	3,23	10)	3,18	11)	2,49
		12)	3,16			7)	2,60
		5)	3,20				
		9)	3,20				

Regression equation: $y = 1,093\ x - 0,352$ mmol/1

$r = 0,987$

the bias figures for all 108 results fell within the range of ± 1,6 SD.

Applying the paired t test for the significance of the difference of mean values by our method and our laboratory, it was found that the differences were not significant ($P > 0,10$) for six of the nine analytes. In three cases the differences were statistically significant, as indicated below.

Analyte	Difference $(\bar{y} - \bar{x})$	S_D	t	Probability
Creatinine (µmol/l)	-6,54	8,56	2,65	$0,05 > P > 0,02$
Albumin (g/l)	1,25	0,67	6,46	$0,001 > P$
Cholesterol (mmol/l)	-0,073	0,057	4,44	$P = 0,001$

It is noteworthy that the two analytes where the highest significant bias occurs for our result relative to our method are the two high molecular weight substances (albumin and cholesterol) where standards of different composition ("pure" analyte or multi-component serum) are likely to be in use in different laboratories.

b) Precision. Between-batch precision shows a satisfactory performance (Table VII), being better than the average figures both for our method and for all methods with the exception of glucose, where the one "wild" result has a serious effect on the calculated SD. Re-calculating the SD on the basis of eleven results only (omitting specimen 2 so that Pool B is reduced from five to four specimens) gives a value of 0,11. This finding demonstrates one

TABLE VII. Between-batch precision of nine selected methods in the Wellcome Quality Control Programme

Assay	All methods		Our method		Our results
	No.	Precision	No.	Precision	Precision
Sodium (mmol/l)	977	2,0	147	1,7	1,2
Potassium (mmol/l)	984	0,11	148	0,098	0,05
Chloride (mmol/l)	847	2,1	43	1,8	1,8
Urea (mmol/l)	1 006	0,71	41	1,0	0,48
Creatinine (µmol/l)	914	15	122	21	7
Glucose (mmol/l)	1 009	0,34	38	0,36	0,40 (0,11)
Albumin (g/l)	807	2,1	54	2,4	0,4
Calcium (mmol/l)	919	0,084	38	0,13	0,059
Cholesterol (mmol/l)	885	0,25	53	0,25	0,05

of the weaknesses of the Wellcome Programme, in that a single discrepant result can have such an untoward influence on the performance-rating.

On the basis of the above findings it appeared satisfactory to employ these nine methods in the experimental portion of this work, together with the methods earlier described for bicarbonate, alkaline phosphatase and aspartate transaminase determination.

CHAPTER 5

5. PERFORMANCE CHARACTERISTICS OF THE ANALYTICAL METHODS AND EQUIPMENT

5.1 Introduction

The application of quantitative clinical chemical findings to medical diagnosis requires the clinician to be able to detect a state of "abnormality" in the numerical values returned by the laboratory on his patient, indicating some associated abnormality in the patient himself. Ideally, this would involve provision to the clinician of information on the "true" level of the analyte under consideration and his comparison of this with a figure for the value to be expected in subjects free from disease. However, such a situation can almost never be realised in practice, for the following reasons:-

1) In the same way that anatomical features (height, body mass, surface area) and physiological observations (pulse rate, body temperature) do not have single, constant values for all subjects, chemical parameters exhibit differing degrees of variability in a healthy population, associated with various predisposing factors. Some of the more obvious of these, for example age and sex, have long been known to influence the "normal range" for many body constituents but the importance of other factors (for example time of day, posture) has only begun to be appreciated in recent years.

2) A number of additional factors, associated with the collection, preservation and transport of specimens, can significantly alter the level of the analyte prior to analysis in the

laboratory, so that the result of the latter operation may not reflect the true situation in the patient.

Several of the factors included under the above two headings are set out in Table VIII.[4]

3) Finally, a degree of inexactness exists in the analytical methods routinely in use in the service departments of clinical chemistry laboratories. Although "definitive" methods are being developed for a number of assays[87] which, by definition, give the best possible measurement of the "true" result, such methods (for example, isotope dilution - mass spectrometry for calcium) are, by their complexity and cost, impracticable for everyday use. Consequently, the methods in routine use represent a compromise between absolute reliability and practicability and can have several sources of imprecision occurring at various stages of the analysis. A number of these, particularly related to colorimetric analysis, are shown in Table IX.[88]

In this chapter the experimental assessment of some of these analytical sources of imprecision is described, as they occur in the equipment and methods for limited function automation already presented. A general discussion of the findings is deferred to the end of the chapter.

5.2 Volumetric precision of sampling devices

5.2.1 Macrovolumes

Volumes of water sampled or dispensed by the various devices in the volume range 0,5 - 5,0 ml were measured gravimetrically on the

TABLE VIII. Factors capable of influencing the result or interpretation of clinical chemical analyses[4]

Factors relating to the patient	Factors relating to the collection, transport and analysis of specimens
1) Age	a) Site of collection
2) Sex	
3) Pregnancy	b) Cleanliness of equipment used to collect and transport specimen
4) Stage of menstrual cycle	
5) Body size	
6) Ethnic group	
7) Place of domicile	
8) Normal diet	c) Interval between collection and analysis of specimen
9) Interval since last meal	
10) Time of day	
11) Recent exercise	d) Conditions of storage during this interval
12) Posture during test	
13) Mental state during test	
14) Non-specific illness	
15) Medication	e) Actual performance of analysis in the laboratory (Table IX)
16) Pathological condition	

TABLE IX. Some sources of variability in colorimetric analysis[88]

Volumetric variability	1) Measurement of specimen, diluent, reagents, filtrates, etc.
	2) Sample carry-over or specimen-diluent interaction.
Chemical variability	3) Inadequate mixing.
	4) Local temperature differences in water bath.
	5) Variable heat transfer through walls of reaction tubes.
	6) Physical or chemical reactions at exposed surfaces (evaporation, oxidation, effect of CO_2, etc.).
	7) Colour instability.
Colorimetric variability	8) Unsteady energy emission from light source (single celled instruments).
	9) Alignment of colorimeter cell.
	10) Response of photocell.
	11) Movement of galvanometer pointer or recorder pen.
	12) Number of significant figures registered.
	13) Carry-over of coloured solution in colorimeter flow-cell or cuvette.

Sartorius 1205 top-loading microbalance, providing a digital display of the measured mass in grammes to three decimal places.

A set of 20 glass test tubes was weighed before and after addition of the dispensed volumes of water. The inherent variability of the balance itself was assessed by 20 replicate weighings of a single tube of each of the two sizes used (10 ml and 20 ml capacity). The results of these weighing experiments are presented in Table X.

Included at the bottom of this table are comparative figures for the working time involved for 20 dispensings by semiautomated devices and conventional mouth-operated pipettes, at three different volumes.

5.2.2 Microvolumes

For measuring the precision of the microvolumes (10 and 20 µl) comprising the actual analytical sample for many of the assays the foregoing gravimetric procedure, having a sensitivity of 0,001 g, would be unsuitable. It was decided, therefore, to base the experimental approach for this part of the work on the dilution of serum containing as an additive some substance of high photometric absorptivity, and for this purpose the dye Evans Blue was selected.

This dye, earlier known by the code name of T-1824, was introduced for use in blood volume measurement by Dawson, Evans and Whipple[89] in 1920. Gregerson and Gibson[90] in 1937 confirmed the suitability of the dye for this purpose, demonstrating the minimal effect of variations in protein and "salt" content and

TABLE X. Assessment of volumetric precision of sampling devices by water-weighing experiments

Measurement	Mean mass (g)	SD	CV %
Single 10 ml test tube	8,6492	0,000513	0,0059
Single 20 ml test tube	15,2795	0,000324	0,0033
20 dispensings of 4,0 ml water			
Oxford Pipettor (1-10 ml)	4,0582	0,01752	0,43
	4,0474	0,00829	0,20
20 dispensings of 2,0 ml water			
Oxford Pipettor (1-10 ml)	2,0571	0,00447	0,22
	2,0387	0,00891	0,45
Oxford Sampler (1-5 ml)	1,9819	0,00934	0,47
	1,9822	0,01066	0,54
Volumetric pipette	1,9488	0,00727	0,37
Oxford Dilutor	2,0702	0,00411	0,20
	2,0494	0,00688	0,34
20 dispensings of 5,0 ml water			
Oxford Sampler (1-5 ml)	4,9429	0,00777	0,16
	4,9608	0,01125	0,23
20 dispensings of 1,0 ml water			
Oxford Pipettor (1-10 ml)	1,1324	0,00971	0,86
	1,1229	0,01128	1,00
Oxford Pipettor (1 ml capacity)	1,0086	0,00411	0,41
	1,0042	0,00271	0,27
Oxford Dilutor	1,0787	0,00362	0,34
	1,0755	0,00252	0,24
Selectapette	1,0112	0,00150	0,15
	1,0044	0,00112	0,11
20 dispensings of 0,5 ml water			
Oxford Pipettor (1,0 ml capacity)	0,4929	0,00133	0,27
	0,4915	0,00115	0,23
Selectapette	0,5002	0,00104	0,21
	0,4994	0,00147	0,29
Graduated pipette (0-1,0 ml)	0,5082	0,00275	0,54
	0,5082	0,00187	0,37

Comparative timings (20 dispensings)

2,0 ml	Volumetric pipette (blown out)	5 minutes	27 seconds
	Oxford Pipettor	1 minute	30 seconds
1,0 ml	Volumetric pipette (blown out)	4 minutes	3 seconds
	Selectapette	1 minute	35 seconds
0,5 ml	Graduated pipette (blown out)	5 minutes	
	Selectapette	1 minute	47 seconds

pH of the plasma on its spectral properties.

For injection into the subject in blood volume studies, Gregerson[91] in 1944 used an aqueous solution of Evans Blue containing 4,5 g/l and found that a 1/500 dilution of such a stock solution with human plasma or serum had an optical density at 624 nm (the absorption peak) of 0,8, measured in a 1 cm cell against a diluent blank.

The preparation used in the present study consisted of 2,0 g Evans Blue (Merck) dissolved in 50 ml of water with magnetic stirring over two hours, followed by filtration on a sintered funnel through Whatman 44 filter paper. The aqueous solution was then diluted 1/11 with pooled human serum by adding 0,4 ml of it to 4,0 ml of serum and mixing on a rotating mixer for two hours. This stock dyed serum was stored at $4^{o}C$ for up to a week with no evidence of change in its optical properties. One millilitre of the dyed serum was diluted to 500 ml with water in a volumetric flask and the absorbance curve of this final dilution measured over the range 450 - 700 nm on the Gilford spectrophotometer against a water blank (Figure 11).

The absorption peak at 610 nm showed some displacement from that recorded by Gregerson,[91] but the absorbance reading at the peak (0,667) was in close agreement with the value of 0,647

$$\left[\frac{40/11}{4,5} \times 0,8\right]$$

calculated from Gregerson's figures. The linearity of absorbance relative to dye concentration was confirmed by preparing 20%, 40%, 60% and 80% subdilutions of the original 1/500 dilution and

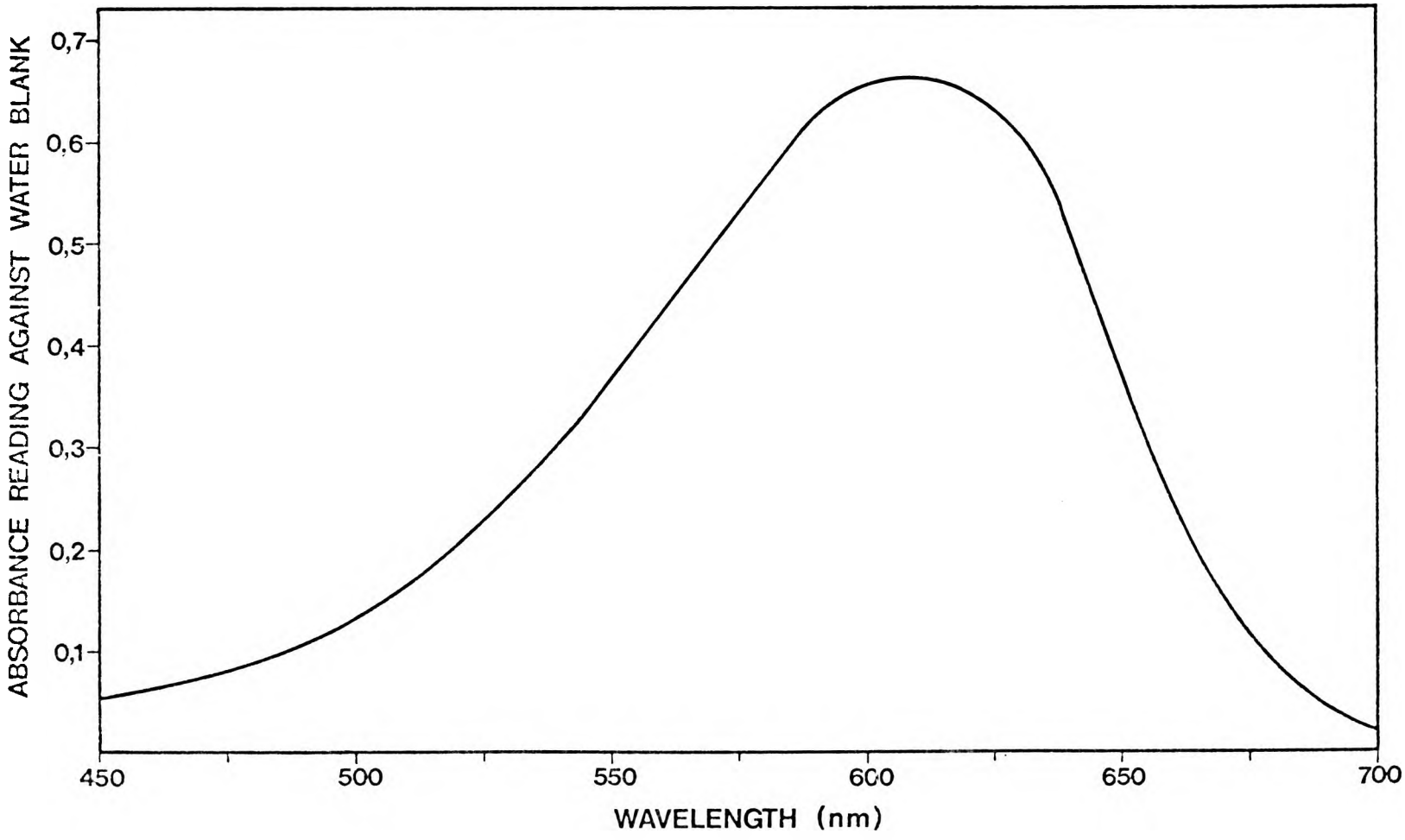

Figure 11. Absorbance curve for Evans Blue/Serum.

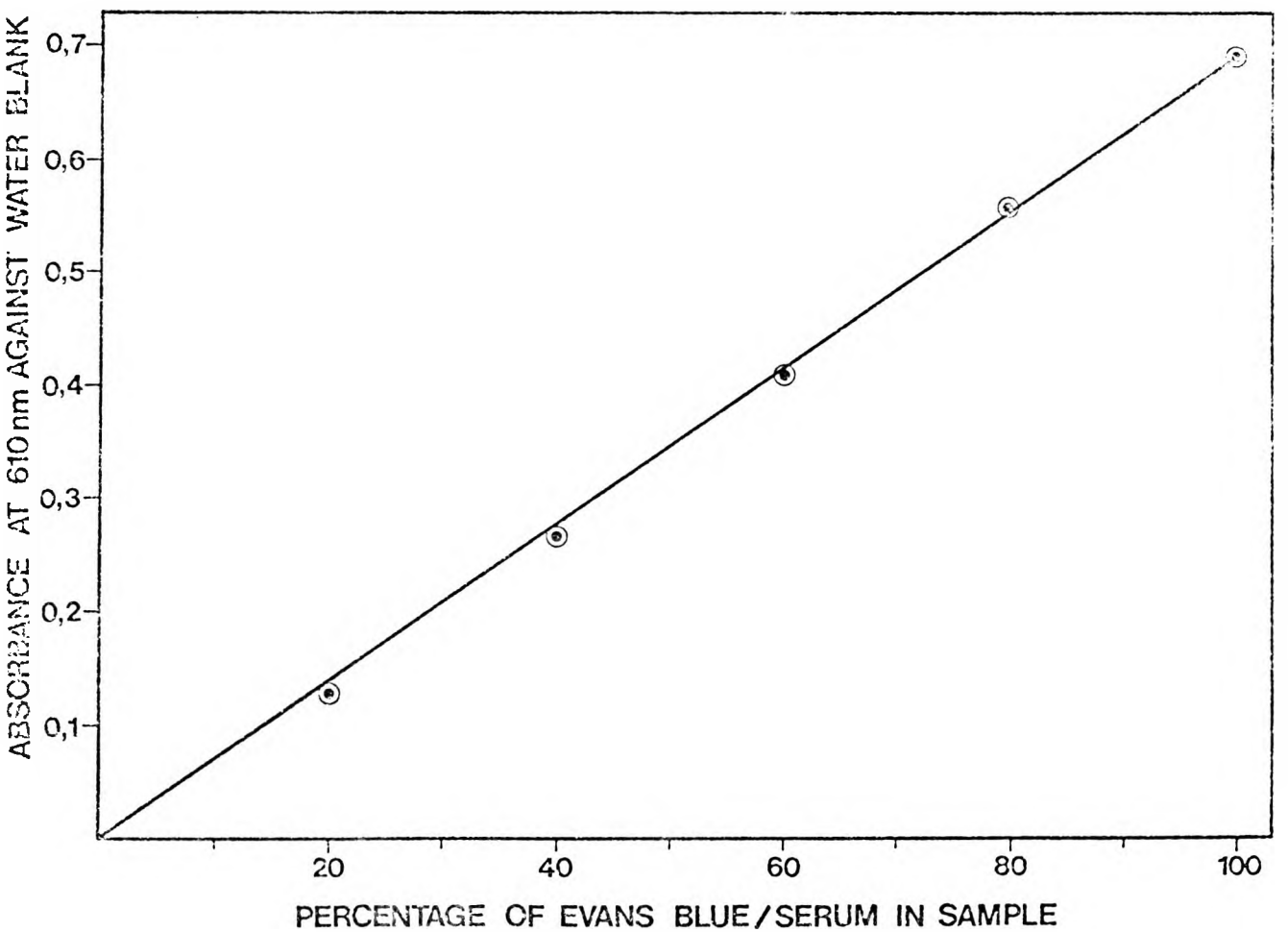

Figure 12. Absorbance at 610 nm in relation to concentration for Evans Blue/Serum.

measuring their absorbance at 610 nm (Figure 12).

For precision studies with the SMI 10 µl Micro/Pettor the stock dyed serum was first diluted 1/5 with pooled serum. A set of 20 dispensings were then made into tubes containing 1 ml of water, measured with the Selectapette. After vortex mixing these dilutions were read on the Gilford at 610 nm, setting to zero on water.

The precisions of the 20 µl volumes from the SMI Micro/Pettor and the Oxford Dilutor were measured in a similar way, using a 1/6 initial dilution of the dyed serum and a water volume of 2,0 ml.

The inherent photometric variability of the measurements was assessed by 20 replicate measurements on "bulk" dilutions, corresponding to the above individual dilutions (0,1 ml dyed serum plus 50 ml of water; 0,05 ml dyed serum plus 30 ml of water), prepared in measuring cylinders.

Data obtained in the foregoing experiments are presented in Table XI.

5.3 Specimen carry-over at time of sampling

When using the Oxford series of samplers it is possible to avoid sample carry-over by using a fresh plastic tip for each separate specimen. The design of the SMI Micro/Pettor, with physical displacement of the sample from the capillary by the Teflon-coated plunger, is intended to obviate this necessity, so that, according to the manufacturers, the same capillary can be used

TABLE XI. Assessment of volumetric precision of microsampling devices by dilution of serum dyed with Evans Blue

Measurement	Mean OD 610 nm	SD	CV %
Photometric precision of readings of diluted Evans Blue serum			
Approximate bulk dilutions:			
1/500	0,6667	0,00114	0,171
	0,6589	0,00117	0,177
1/600	0,5516	0,000821	0,146
	0,5769	0,00136	0,235
	0,5715	0,00132	0,230
MEAN		0,00116	0,192
20 dispensings of 10 µl (SMI)	0,6688	0,00512	0,77
	0,6692	0,00478	0,72
20 dispensings of 20 µl (SMI)	0,5066	0,00410	0,81
	0,5253	0,00485	0,92
20 dispensings of 20 µl (Oxford Dilutor)	0,5042	0,00578	1,15
	0,5120	0,00621	1,21

for up to 3 000 dispensings.

In order to assess the amount of carry-over which does occur, the transfer of radioactivity of I^{125}-labelled thyroxine was measured.

For this purpose a vial initially containing 200 microcuries (7,4 megabecquerel) of L-[3´5´-I^{125}] thyroxine in 1 ml of 50% aqueous propylene glycol was obtained from the Radiochemical Centre, Amersham. On an initial activity check 20 µl of a 1/10 aqueous dilution of this material, added to 1 ml of water in a counting vial, gave 522 020 counts per minute on the Packard 300-Sample Auto-Gamma Counter.

The carry-over study was performed using the principle described by Broughton *et al.*[92] in 1969. The labelled thyroxine was diluted 1/10 with pooled serum and 0,3 ml of this dilution dispensed into each of three plastic tubes ("high" level). Into three further tubes was pipetted 1,0 ml of the unlabelled pooled serum ("low" level). Using the particular sampler under test, 10 or 20 µl volumes of the three "high" samples were pipetted into counting vials containing 1 ml of water, followed by dispensings from the three "low" samples. The outside of the pipette tip was wiped dry with adsorbant tissue between samplings. The vials were then counted for 1 minute on the Gamma counter.

Percentage carry-over was calculated from the formula[92]

$$\frac{L_1 - L_3}{H_3 - L_3}$$

where L and H denote the low or high activity sample counts and

the suffix is the sampling number out of the sequence of three vials.

The relevant activity measurements and derived carry-over figures are given in Table XII.

5.4 Photometric precision of readings made in the Gilford flow-cell

5.4.1 Comparison of the photometric precisions and working times for 20 replicate samplings using the flow-cell and a conventional colorimeter cuvette (Table XIII)

For this experiment the solution subjected to photometric measurement was the inorganic grey solution described by Thomson[93] in 1946, prepared with the following composition:-

$Cr_2(SO_4)_3, K_2SO_4, 24H_2O$	16,67	g/l
$CuSO_4, 5H_2O$	33,33	g/l
$CoSO_4, (NH_4)_2SO_4, 6H_2O$	39,5	g/l
$K_2Cr_2O_7$	0,120	g/l

After preparation, this solution was allowed to "age" for six weeks in a dark glass bottle to obtain a final stable grey colour.

Sets of twenty readings at 520 nm on this solution were made with the Gilford and with the Leitz Model M Photrometer, using with the latter a glass cuvette removed from the colorimeter for filling and emptying. In order to obtain absorbance readings on

TABLE XII. Radiometric measurement of sampling carry-over

Sampling device		Counts per minute $\frac{L_1 - L_3}{H_3 - L_3}$	Percentage carry-over
10 µl SMI	(1)	$\frac{252 - 44}{256875 - 44}$	0,081
	(2)	$\frac{263 - 34}{247333 - 34}$	0,093
	(3)	$\frac{159 - 55}{253103 - 55}$	0,041
	(4)	$\frac{228 - 6}{251977 - 6}$	0,088
			Mean 0,076
20 µl SMI	(1)	$\frac{308 - 81}{494454 - 81}$	0,046
	(2)	$\frac{428 - 247}{503066 - 247}$	0,036
	(3)	$\frac{352 - 74}{493937 - 74}$	0,056
	(4)	$\frac{412 - 125}{494532 - 125}$	0,058
			Mean 0,049
20 µl Oxford	(1)	$\frac{12235 - 669}{537389 - 669}$	2,15
	(2)	$\frac{14887 - 862}{541112 - 862}$	2,60
	(3)	$\frac{14387 - 486}{548077 - 486}$	2,58
	(4)	$\frac{12439 - 310}{537015 - 310}$	2,27
			Mean 2,40

TABLE XIII. Readings of absorbance of Neutral Grey solution on the Leitz colorimeter and the Gilford flow-through absorptionmeter at 520 nm against water (20 samplings)

		Mean OD	SD	CV %	Reading time
A)	LEITZ	0,7417	0,00353	0,48	9 minutes 18 seconds
	GILFORD	0,7986	0,00196	0,25	1 minute 54 seconds
B)	LEITZ	0,4219	0,00285	0,68	8 minutes 0 seconds
	GILFORD	0,4594	0,00310	0,67	1 minute 50 seconds
C)	LEITZ	0,7327	0,00258	0,35	8 minutes 25 seconds
	GILFORD	0,7953	0,00177	0,22	2 minutes 30 seconds
D)	LEITZ	0,1828	0,00199	1,09	8 minutes 5 seconds
	GILFORD	0,2035	0,00190	0,93	2 minutes 24 seconds

Mean values: LEITZ CV 0,65 in 8 minutes 27 seconds
GILFORD CV 0,52 in 2 minutes 10 seconds

the Leitz at the same degree of sensitivity as the Gilford (0,001 absorbance units), the output from the Leitz photocell was fed to an external digital reader (Mediconics "Chemputer-100") giving a display of the absorbance in a form corresponding to that of the Gilford.

In order to measure the precision at absorbance values lower than that of the original grey solution, readings were also made on dilutions of the latter of approximately 50% and 25% concentration (Table XIII, "B" and "D").

5.4.2 Photometric precision of readings on the final coloured solutions for the various methods

A "bulk" preparation of the final coloured product for the seven colorimetric assays (urea, creatinine, glucose, albumin, calcium, cholesterol and alkaline phosphatase) was prepared by scaling up the volumes of specimen and reagents to give a final volume of around 20 ml. In the case of calcium, for example, the bulk solution was prepared by mixing in a conical flask 10 ml of the CPC reagent, 0,2 ml of the aqueous standard (2,50 mmol/l) and 10 ml of the buffer reagent. The analyte concentrations for the other bulk solutions were as shown in Table XIV.

Each final mixture, after the appropriate colour development time for the particular test, was sampled ten times on the Gilford, setting to zero on the normal reagent blank. After an interval of 15 minutes the remaining portion of the bulk preparation was resampled in the same way.

TABLE XIV. Photometric precision and stability of readings on final solutions

Analyte	Matrix and concentration	Initial OD Mean and CV	15 minute OD Mean and CV	Drift per minute
Urea	SMA serum 21,5 mmol/l	0,4334 0,40%	0,4234 0,32%	-0,15%
Creatinine	Aqueous 500 µmol/l	0,8843 0,50%	0,9560 0,35%	+0,54%
Glucose	Aqueous 10 mmol/l	0,6634 0,08%	0,6404 0,17%	-0,23%
Albumin	SMA serum 46 g/l	0,2609 1,56%	0,2591 0,95%	-0,05%
Calcium	Aqueous 2,50 mmol/l	0,4448 0,45%	0,4419 0,27%	-0,04%
Cholesterol	SMA serum 4,46 mmol/l	0,2774 0,19%	0,2753 0,18%	-0,05%
Alkaline phosphatase	TQC serum (abnormal) 82 U/l (Phosphastrate)	0,8837 0,11%	0,8790 0,12%	-0,04%

Values for the initial and final mean absorbance readings, their standard deviation and the change occurring during the 15 minute period are presented in Table XIV.

5.4.3 Carry-over of the final coloured solutions in the Gilford flow-cell

The experimental protocol adopted was similar to that described earlier for sampling carry-over, involving the measurement in sequence of three high and three low levels.

Bulk preparations of the final coloured products were prepared as in the previous experiment, but having in some cases somewhat higher analyte levels so as to maximise the measured carry-over. Corresponding bulk preparations of the reagent blank ("low" readings) were also made in each case. Three samplings of the high level were followed by three samplings of the low level and these sequences each repeated three times. The mean absorbance differences and percentage carry-over are shown in Table XV.

5.5 Within-batch precision and correlation of aqueous and serum-based standards

The overall precision, as indicated by the final instrumental reading, for the various calibrating standards was determined by carrying out ten replicate assays of each. In the case of sodium, potassium and albumin assays only the single standard used for both manual and automated assays was investigated; for the other analytes both the aqueous standards used for the manual methods and the serum-based standards used on the SMA 6/60

TABLE XV. Carry-over in Gilford flow-cell of final solutions

Analyte	"High" level	"Low" level	Mean absorbances $\frac{L_1 - L_3}{H_3 - L_3}$	Mean carry-over
Urea	Calibrate QC 27,8 mmol/l	Reagent blank	$\frac{0,005}{0,532}$	0,9%
Creatinine	Aqueous 400 µmol/l	Reagent blank	$\frac{0,0023}{0,717}$	0,3%
Glucose	SMA serum 14 mmol/l	Reagent blank	$\frac{0,0020}{0,650}$	0,3%
Albumin	SMA serum 46 g/l	Reagent blank	$\frac{0,0010}{0,260}$	0,4%
Calcium	Aqueous 2,5 mmol/l	Reagent blank	$\frac{0,0037}{0,442}$	0,8%
Cholesterol	Ortho QC 8,47 mmol/l	Reagent blank	$\frac{0,0050}{0,491}$	1,0%
Alkaline phosphatase	Versatol QC 120 U/l	Reagent blank	$\frac{0,0070}{1,319}$	0,5%

or 12/60 were analysed. The relative values of the latter were then calculated (Table XVI).

For sodium, potassium and chloride the values entered under "mean reading" in Table XVI are the direct concentration display of the flame photometer or chloride meter. For bicarbonate the figures given are the gas volumes read off the scale on the Harleco capillary, while for the remaining six assays the readings are the absorbances read on the Gilford.

5.6 Between batch precision and correlation with fully automated procedures

Having investigated certain of the factors associated with the separate stages of several of the selected methods, the final and major undertaking was to investigate their overall performance in the analysis of patients' specimens.

For this purpose, 20 unselected specimens that had been analysed on the automatic analysers were collected from the Johannesburg General Hospital Laboratory and analysed in two batches of ten by the manual methods on the afternoon of the same day. After storage at $4^{o}C$ overnight the 20 samples were then re-analysed the next morning.

Only one analyte was investigated on any single set of patients' specimens, so the experimental work for this study was spread over four weeks, at the rate of three analyte studies per week.

The between-batch duplicated results, together with the

TABLE XVI. Within-batch precision and correlation of aqueous and serum-based standards

Analyte	Matrix and concentration		Mean reading		CV	Serum value read or calculated from aqueous standard
Sodium	Lock-in serum	140 mmol/l	140,45	mmol/l	0,32%	-
Potassium	Lock-in serum	5,4 mmol/l	5,377	mmol/l	0,64%	-
Chloride	Aqueous	100 mmol/l	99,6	mmol/l	0,52%	
	Lock-in serum	103 mmol/l	102,1	mmol/l	0,31%	102,1 mmol/l
Bicarbonate	Aqueous	25 mmol/l	30,88	divisions	4,4%	
	Lock-in serum	26 mmol/l	31,78	divisions	4,7%	25,7 mmol/l
			Mean absorbance			
Urea	Aqueous	10 mmol/l	0,1997		1,21%	
	Lock-in serum	21,1 mmol/l	0,4242		1,04%	21,24 mmol/l
Creatinine	Aqueous	200 µmol/l	0,3682		1,00%	
	Lock-in serum	477 µmol/l	0,8516		1,20%	463 µmol/l
Glucose	Aqueous	10 mmol/l	0,6432		0,13%	
	SMA serum	14 mmol/l	0,8435		0,25%	13,1 mmol/l
Albumin	SMA serum	46 g/l	0,2618		1,49%	-
Calcium	Aqueous	2,50 mmol/l	0,4219		1,24%	
	SMA serum	2,25 mmol/l	0,3898		1,27%	2,31 mmol/l
Cholesterol	Isopropanol solution	5,18 mmol/l	0,3503		1,17%	
	SMA serum	3,42 mmol/l	0,2126		0,63%	3,14 mmol/l

results by the automated analysers and simple correlation data are presented in Table XVII (a - o), while statistical analysis of the paired manual results appears in Table XVIII.

In addition to the methods previously described, the following additional manual procedures for urea and cholesterol were carried out:-

5.6.1 Urea estimation by a modified Berthelot method[94,95]

Principle: The ammonia formed from urea by the action of urease reacts with hypochlorite to form chloramine which, in turn, reacts with salicylate in the presence of nitroprusside as catalyst to form a green coloured indophenol compound.

Reagents: A reagent kit based on the above procedure (but lacking exact specifications on reagent composition) was obtained locally from Clinical Science Laboratories.

Standard: An aqueous solution of urea containing 10 mmol/l, as used with the diacetyl monoxime method.

Procedure: Pipette into a set of 10 x 1,5 cm tubes:

1,0 ml urease solution,

20 µl specimen or standard (no addition is made to the blank).

Vortex mix, then incubate at 37°C for 15 minutes.

Following incubation, add:

2,5 ml salicylate reagent,

2,5 ml alkaline hypochlorite reagent.

Mix and incubate at 37°C for 5 minutes.

Cool to room temperature and read on the Gilford against the reagent blank at 620 nm.

TABLE XVII. Between-batch precision and correlation with fully automated methods

(a) SODIUM (mmol/l)

No.	6/60 result (x)	Manual results (a)	(b)	Alternately rounded mean (y)
1	131	130	131	131
2	136	137	136	136
3	136	137	138	138
4	139	138	140	139
5	139	138	140	139
6	139	139	138	138
7	139	141	141	141
8	140	138	139	139
9	140	138	138	138
10	141	141	142	141
11	141	141	139	140
12	141	139	139	139
13	141	142	142	142
14	141	138	139	139
15	141	140	141	140
16	142	141	142	142
17	142	143	142	142
18	142	141	140	141
19	142	141	141	141
20	146	144	146	145
Mean	139,95	139,35	139,70	139,55

Regression equation: $y = 0{,}861\ x + 19{,}12$

$r = 0{,}926$

TABLE XVII continued

(b) POTASSIUM (mmol/l)

No.	6/60 result (x)	Manual results (a)	(b)	Alternately rounded mean (y)
1	3,2	3,1	3,1	3,1
2	3,4	3,5	3,3	3,4
3	3,7	3,5	3,7	3,6
4	3,7	3,6	3,8	3,7
5	3,9	3,8	3,9	3,9
6	3,9	3,9	3,9	3,9
7	3,9	3,5	3,6	3,5
8	4,0	4,0	4,0	4,0
9	4,1	3,9	3,9	3,9
10	4,1	4,2	4,4	4,3
11	4,2	4,1	4,1	4,1
12	4,2	4,1	4,1	4,1
13	4,3	4,2	4,2	4,2
14	4,3	4,1	4,1	4,1
16	4,4	4,3	4,3	4,3
17	4,4	4,3	4,3	4,3
18	4,6	4,5	4,5	4,5
19	4,6	4,4	4,4	4,4
20	5,7	5,4	5,5	5,5
Mean	4,145	4,025	4,060	4,045

Regression equation: $y = 0{,}931\ x + 0{,}185$

$r = 0{,}972$

TABLE XVII continued

(c) CHLORIDE (mmol/l)

No.	6/60 result (x)	Manual results (a)	(b)	Alternately rounded mean (y)
1	87	89	92	91
2	95	94	97	95
3	97	99	97	98
4	98	101	101	101
5	98	100	100	100
6	99	101	101	101
7	101	103	104	104
8	101	103	104	103
9	102	102	102	102
10	102	103	102	103
11	103	103	104	103
12	103	106	106	106
13	103	105	105	105
14	103	106	104	105
15	104	107	104	106
16	105	109	109	109
17	108	107	107	107
18	108	112	112	112
19	112	114	116	115
20	116	118	116	117
Mean	102,25	104,10	104,15	104,15

Regression equation: $y = 0{,}972\ x + 4{,}79$

$r = 0{,}973$

TABLE XVII continued

(d) BICARBONATE (mmol/l)

No.	6/60 result (x)	Manual results (a)	(b)	Alternately rounded mean (y)
1	18	16,1	15,7	16
2	19	18,4	18,8	19
3	22	22,3	22,3	22
4	23	22,8	22,0	22
5	24	22,8	22,0	22
6	24	23,2	23,2	23
7	25	24,1	23,2	24
8	25	23,2	23,6	23
9	25	24,1	24,1	24
10	26	24,3	21,9	23
11	26	29,9	23,5	24
12	26	24,7	25,1	25
13	26	24,5	24,5	25
14	27	25,4	26,2	26
15	27	27,1	26,2	27
16	29	25,9	25,1	25
17	29	28,2	28,2	28
18	29	27,1	28,4	28
19	32	28,2	29,0	29
20	32	30,9	30,1	31
Mean	25,70	24,36	24,155	24,30

Regression equation: $y = 0{,}910\ x + 0{,}92$

$r = 0{,}957$

TABLE XVII continued

(c) UREA (mmol/l) : DIACETYL MONOXIME

No.	6/60 result (x)	Manual results (a)	(b)	Alternately rounded mean (y)
1	3,0	3,4	2,8	3,1
2	3,5	3,7	3,3	3,5
3	3,5	3,1	3,5	3,3
4	3,7	4,0	3,4	3,7
5	3,8	3,8	3,9	3,9
6	4,6	5,4	4,6	5,0
7	5,5	5,2	5,3	5,2
8	5,5	5,6	5,8	5,7
9	6,0	5,8	5,3	5,6
10	6,0	6,0	5,8	5,9
11	6,1	6,1	6,2	6,1
12	7,5	7,5	7,4	7,5
Mean	4,89	4,97	4,78	4,88
13	9,5	9,4	9,2	9,3
14	12,0	12,0	11,6	11,8
15	12,6	12,4	12,3	12,3
16	13,9	13,5	13,0	13,3
17	18,9	18,2	17,8	18,0
18	23,2	22,4	21,7	22,0
19	24,2	22,7	22,7	22,7
20	35,2	31,0	31,6	31,3
(Overall) Mean	10,41	10,06	9,86	9,96

Regression equation (all results): $y = 0,901\,x + 0,58$

$r = 0,999$

TABLE XVII continued

(f) UREA (mmol/l) : BERTHELOT

No.	6/60 result (x)	Manual results (a)	(b)	Alternately rounded mean (y)
1	3,0	3,0	3,3	3,2
2	3,5	3,7	3,9	3,8
3	3,5	4,2	3,7	3,9
4	3,7	4,3	4,2	4,3
5	3,8	3,9	4,0	3,9
6	4,6	4,9	5,0	5,0
7	5,5	6,0	5,8	5,9
8	5,5	5,7	5,5	5,6
9	6,0	5,3	5,4	5,3
10	6,0	6,6	6,0	6,3
11	6,1	6,2	6,5	6,4
12	7,5	7,1	7,4	7,2
Mean	4,89	5,08	5,06	5,07
13	9,5	9,9	10,0	10,0
14	12,0	12,0	12,4	12,2
15	12,6	12,2	12,3	12,2
16	13,9	13,9	13,9	13,9
17	18,9	19,6	20,0	19,8
18	23,2	21,7	22,3	22,0
19	24,2	24,1	24,0	24,1
20	35,2	31,7	32,3	32,0
(Over-all) Mean	10,41	10,30	10,40	10,35

Regression equation (all results): $y = 0,928\ x + 0,69$

$r = 0,997$

TABLE XVII continued

(g) CREATININE (µmol/l)

No.	6/60 result (x)	Manual results (a)	(b)	Alternately rounded mean (y)
1	69	81	81	81
2	75	89	97	93
3	83	79	85	82
4	89	90	87	89
5	93	93	102	97
6	103	100	105	103
7	103	104	102	103
8	109	122	120	121
9	109	117	119	118
10	118	115	112	113
11	118	130	130	130
12	119	153	140	147
Mean	99,00	106,08	106,67	106,42
13	131	130	134	132
14	147	146	148	147
15	149	155	161	158
16	179	187	182	184
17	185	201	210	206
18	226	231	228	229
19	231	220	235	228
20	484	429	438	433
(Over-all) Mean	146,00	148,60	150,80	149,70

Regression equation (all results): $y = 0,873\,x + 22,21$

$r = 0,992$

TABLE XVII continued

(h) GLUCOSE (mmol/l)

No.	12/60 result (x)	Manual results (a)	(b)	Alternately rounded mean (y)
1	3,9	3,72	3,66	3,7
2	4,0	3,89	3,55	3,7
3	4,1	3,77	3,89	3,8
4	4,2	4,11	4,44	4,3
5	4,2	3,89	3,89	3,9
6	4,8	4,77	4,77	4,8
7	5,3	5,11	5,38	5,2
8	5,6	5,11	5,00	5,1
9	5,7	5,49	5,77	5,6
10	5,9	5,88	5,38	5,6
11	6,1	5,88	6,66	6,3
12	6,3	5,94	5,99	7,0
Mean	5,01	4,80	4,87	4,83
13	6,7	6,99	6,66	6,8
14	7,4	7,27	7,10	7,2
15	7,8	8,10	8,16	8,1
16	8,0	7,71	7,88	7,8
17	9,1	8,66	8,60	8,6
18	12,6	13,10	12,71	12,9
19	17,8	18,20	18,43	18,3
20	18,1	19,48	19,92	19,7
Mean	7,38	7,35	7,39	7,37

Regression equation (all results): $y = 1{,}085\,x - 0{,}64$

$r = 0{,}998$

TABLE XVII continued

(j) ALBUMIN (g/l)

No.	12/60 result (x)	Manual results (a)	(b)	Alternately rounded mean (y)
1	20	23,0	22,8	23
2	24	24,6	24,8	25
3	27	28,4	27,9	28
4	29	29,2	29,9	30
5	30	31,6	32,0	32
6	34	37,7	37,4	38
7	35	35,9	36,9	36
8	35	35,7	36,2	36
9	37	37,5	37,3	37
10	40	38,4	38,9	39
11	40	39,1	39,1	39
12	42	41,0	43,5	42
13	42	41,9	42,0	42
14	42	40,7	41,2	41
15	44	42,3	45,0	44
16	45	41,6	42,0	42
17	46	46,0	45,7	46
18	48	46,0	47,4	47
19	48	45,9	46,3	46
20	52	49,5	47,8	49
Mean	38,00	37,80	38,21	38,10

Regression equation: $y = 0{,}840\ x + 6{,}17$

$r = 0{,}989$

TABLE XVII continued

(k) CALCIUM (mmol/l)

No.	12/60 result (x)	Manual results (a)	(b)	Alternately rounded mean (y)
1	1,97	2,04	2,03	2,04
2	1,99	1,98	1,99	1,98
3	2,01	2,06	2,04	2,05
4	2,05	1,88	1,90	1,89
5	2,06	2,03	2,02	2,03
6	2,10	2,03	1,98	2,00
7	2,11	2,08	2,02	2,05
8	2,16	2,11	2,04	2,08
9	2,17	2,21	2,14	2,17
10	2,21	2,28	2,21	2,25
11	2,22	2,23	2,12	2,17
12	2,25	2,26	2,28	2,27
13	2,25	2,29	2,21	2,25
14	2,27	2,27	2,20	2,24
15	2,27	2,05	2,05	2,05
16	2,28	2,21	2,32	2,26
17	2,40	2,11	2,26	2,19
18	2,41	2,34	2,30	2,32
19	2,42	2,41	2,32	2,36
20	2,47	2,56	2,51	2,54
Mean	2,204	2,172	2,147	2,160

Regression equation: $y = 0,854\ x + 0,194$

$r = 0,854$

TABLE XVII continued

(1) CHOLESTEROL (mmol/l) : ENZYMATIC

No.	12/60 result (x)	Manual results (a)	(b)	Alternately rounded mean (y)
1	2,41	2,33	1,93	2,13
2	2,95	3,04	3,08	3,06
3	3,10	3,50	3,31	3,41
4	3,16	3,20	3,27	3,23
5	3,22	3,37	3,54	3,46
6	3,47	3,35	3,54	3,44
7	4,45	4,35	4,51	4,43
8	4,72	4,79	4,86	4,83
9	4,88	4,55	4,78	4,66
10	5,00	5,07	5,26	5,17
11	5,11	5,50	5,47	5,48
12	5,12	4,62	4,86	4,74
13	5,52	5,47	5,66	5,57
14	5,83	5,98	6,24	6,11
15	5,90	5,63	5,77	5,70
16	6,04	5,72	5,96	5,84
17	6,15	6,30	6,31	6,30
18	6,26	6,00	6,15	6,08
19	6,40	6,50	6,49	6,49
20	7,00	6,81	6,64	6,73
Mean	4,835	4,804	4,882	4,843

Regression equation: $y = 0{,}967\ x + 0{,}170$

$r = 0{,}987$

TABLE XVII continued

(m) CHOLESTEROL (mmol/l) : LIEBERMANN-BURCHARD

No.	12/60 result (x)	Manual results (a)	(b)	Alternately rounded mean (y)
1	2,41	2,67	2,79	2,73
2	2,95	3,36	3,62	3,49
3	3,10	3,32	3,32	3,32
4	3,16	3,59	3,27	3,43
5	3,22	3,77	3,57	3,67
6	3,47	3,56	3,71	3,64
7	4,45	5,02	4,74	4,88
8	4,72	5,20	5,43	5,31
9	4,88	5,45	4,86	5,16
10	5,00	5,89	5,37	5,63
11	5,11	5,53	6,04	5,78
12	5,12	5,16	4,84	5,00
13	5,52	5,45	5,46	5,46
14	5,83	6,90	6,38	6,64
15	5,90	6,30	6,01	6,15
16	6,04	5,92	5,39	5,66
17	6,15	6,22	6,43	6,32
18	6,26	6,95	6,72	6,84
19	6,40	6,96	7,24	7,10
20	7,00	7,51	7,75	7,63
Mean	4,835	5,237	5,147	5,192

Regression equation: $y = 1{,}015\ x + 0{,}284$

$r = 0{,}976$

TABLE XVII continued

(n) ALKALINE PHOSPHATASE (U/l at 37°C)

No.	12/60 result (x)	Manual results (a)	(b)	Alternately rounded mean (y)
1	39	14,5	14,9	15
2	54	19,9	19,6	20
3	63	20,8	21,7	21
4	65	23,2	27,0	25
5	82	26,9	26,5	27
6	84	22,3	22,5	22
7	87	28,6	28,4	29
8	93	34,5	35,2	35
Mean	70,88	23,84	24,48	24,25
9	102	35,7	36,9	36
10	109	40,8	41,8	41
11	112	42,8	41,7	42
12	112	45,8	45,7	46
13	128	35,8	35,7	36
14	187	66,5	71,6	69
15	208	61,4	59,5	60
16	233	64,1	64,1	64
17	241	69,8	68,7	69
18	255	77,3	75,4	76
19	300	88,3	87,3	88
20	580	141,7	140,1	141
(Over-all) Mean	156,70	48,04	48,22	48,10

Regression equation (all results): $y = 0{,}238\,x + 10{,}79$

$r = 0{,}982$

TABLE XVII continued

(o) ASPARTATE TRANSAMINASE

No.	12/60 result (U/l) (x)	Manual results (Δ OD/minute)		Rounded mean (U/l) (y)
	37°C	(a)	(b)	30°C
1	8	0,0060	0,0060	8
2	9	0,0085	0,0080	11
3	10	0,0060	0,0070	8
4	10	0,0080	0,0070	10
5	11	0,0090	0,0080	11
6	11	0,0095	0,0100	13
7	12	0,0100	0,0100	13
8	12	0,0100	0,0090	12
9	13	0,0090	0,0105	13
10	21	0,0155	0,0155	20
11	21	0,0135	0,0110	16
12	21	0,0155	0,0150	20
Mean	13,25	0,01004	0,00975	12,92
13	43	0,0195	0,0205	26
14	45	0,0235	0,0235	30
15	46	0,0230	0,0220	29
16	63	0,0290	0,0315	39
17	73	0,0335	0,0345	44
18	79	0,0370	0,0390	49
19	85	0,0395	0,0400	51
20	292	0,1345	0,1345	173
(Over-all) Mean	44,25	0,02300	0,02313	29,80

Regression equation (all results): $y = 0,571\,x + 4,55$

$r = 0,999$

TABLE XVIII. Statistical analysis of between-batch precision data (Table XVII)

Test	Net sign (b - a)	Σ (b - a) (SI units)	"t"	SD of duplicates (SI units)	Corrected SD (SI units)	Mean level (SI units)	CV %
Sodium	+5/20	+0,35	1,38	0,822	0,784	139,55	0,56
Potassium	+5/20	+0,035	1,68	0,0689	0,0643	4,045	1,59
Chloride	+1/20	+0,05	0,14	1,084	1,083	104,15	1,04%
Bicarbonate	-3/20	-0,205	1,11	0,585	0,567	24,30	2,33
Urea: Diacetyl method							
1-12	-2/12	-0,192	1,74	0,292	0,259	4,88	5,31
1-20	-7/20	-0,200	2,36	0,297	0,261	9,96	2,62
Urea: Berthelot method							
1-12	+2/12	+0,0167	0,19	0,208	0,207	5,07	4,08
1-20	+7/20	+0,0950	1,33	0,230	0,220	10,35	2,13
Creatinine							
1-12	0/12	+0,58	0,33	4,11	4,09	106,42	3,84
1-20	+4/20	+2,20	1,53	4,70	4,44	149,70	2,97
Glucose							
1-12	+2/12	+0,068	0,71	0,230	0,225	4,83	4,66
1-20	+2/20	+0,039	0,56	0,214	0,212	7,37	2,88
Albumin	+7/20	+0,405	1,84	0,736	0,678	38,10	1,78
Calcium	-9/20	-0,0245	1,68	0,0481	0,0449	2,160	2,08
Cholesterol: Enzymatic	+10/20	+0,0775	2,02	0,1302	0,1181	4,843	2,44
Liebermann-Burchard	-1/20	-0,0895	1,21	0,2630	0,2274	5,192	4,38
Alkaline phosphatase							
1- 8	+3/8	+0,638	1,32	1,01	0,90	24,25	3,71
1-20	-1/20	+0,180	0,46	1,20	1,14	48,10	2,37
Aspartate transaminase		(OD units)		(OD units)	(OD units)	(OD units)	
1-12	-3/12	-0,00029	0,96	0,00074	0,00071	0,00990	7,2
1-20	+1/20	+0,00013	0,47	0,00082	0,00081	0,02306	3,5

5.6.2 Cholesterol estimation by a modified Liebermann-Burchard procedure

Principle: In this direct procedure, introduced by Watson[79] in 1960, the Liebermann-Burchard reaction is carried out in the presence of an acetic acid solution of 2,5 dimethyl-benzene sulphonic acid, in which medium protein is dissolved by the Liebermann-Burchard reagents.

Reagents: A commercial kit based on this procedure was obtained from Roche Diagnostica, providing reagents with the following composition:-

a) 2,5 dimethylbenzene sulphonic acid 50 mmol/l; acetic acid 6,9 mol/l; acetic anhydride 6,3 mol/l.

b) Cholesterol standard, 5,18 mmol/l (200 mg/100 ml) in glacial acetic acid.

Additional reagent: Concentrated sulphuric acid.

Procedure: Pipette into a set of 10 x 1,5 cm tubes, held in a water bath at room temperature:

2,5 ml of the acid reagent mixture (a),
0,1 ml serum or standard.
(0,1 ml distilled water is added to the blank).
Vortex mix and allow to stand for 15 minutes.

Then add:

0,5 ml concentrated sulphuric acid, dropwise with shaking, so as to dissolve up the precipitate which forms.
After 20 minutes remove the tubes from the water bath and read the green coloured solutions on the Gilford at 580 nm.

5.7 Discussion of results

5.7.1 Terminology

The term "precision" in relation to quantitative analysis was defined by Vogel[96] as "the concordance of a series of measurements of the same quantity". This attribute can be expressed numerically as either the standard deviation or percentage coefficient of variation of the set of numbers obtained.

Although it has recently been advocated[97] that these measured parameters of analytical performance in Clinical Chemistry should rather be referred to as "imprecision", a degree of tortuosity can arise when the terms "precision" and "imprecision" are used descriptively, as they frequently are in the present work. Thus such direct and well-understood phrases as "the method shows improved precision" or "the precision required for clinical purposes" would need to be changed to "the method shows decreased imprecision" and "the imprecision tolerable for clinical purposes" (a rather negative concept).

Consequently, in this work the term precision is retained for the above numerical indices of variability, while the descriptive terms precision and imprecision (or variability) are, in general, used when discussing respectively the virtues or imperfections of an analytical method.

5.7.2 Volumetric precision

5.7.2.1 Interpretation of statistical data

When carrying out relatively small numbers of replicate measurements for the determination of the standard deviation, the presence

or absence of a single deviant reading can significantly affect the final result. For example, ten replicate measurements of a parameter at a level of around 100 arbitrary units might produce the following results:-

Set A	10 x 100	CV = 0%
Set B	9 x 100; 99	CV = 0,317%
Set C	8 x 100; 99; 101	CV = 0,471%

In determining the within-day precision of urea and electrolyte estimations by ten replicate daily analyses on each of ten days, Campbell and Owen[98] found the following variation in the daily CVs:-

Sodium	0,9 - 3,1%
Potassium	0,9 - 3,6%
Chloride	0,8 - 3,5%
Urea	1,0 - 4,6%

The differences in value between several of the pairs of duplicated CV estimations (Tables X, XI) demonstrate the same effect.

5.7.2.2 Additional sources of variability

As a result of the additive property of variance (SD^2) the overall measured SD, in cases where there are a number of independent sources of variability, will be given by the expression[99]

$$SD\ (total) = \sqrt{(SD_1)^2 + (SD_2)^2 + \ldots.(SD_N)^2}$$

The term CV can be substituted for SD in the above expression, to allow for the contributory sources of variability having different units of measurement (volume, mass, absorbance reading,

etc.).

In a simple case where only two sources of variability exist, and where the overall SD, together with that of one of the sources, can be measured, it is possible to calculate the residual variability attributable to the other source. This is illustrated in the following table, where the numbers denote arbitrary units:-

Overall SD = 10

SD for source A	Residual SD for source B
10	0
9	4,36
8	6
7	7,14
6	8
5	8,66
4	9,17
3	9,54
2	9,80
1	9,95

Consequently, if one of the two sources of variability has an SD (or CV) not exceeding half that of the total SD, the true SD of the second source will be not less than 87% of the total SD.

The above considerations are relevant to the results for the volumetric precision experiments shown in Tables X and XI. In the case of the gravimetric experiments the inherent SD of the balance itself (averaging around 0,0004 g) was less than half the smallest overall SD measured (0,00104 g for the dispensing of 0,5 ml by the Selectapette). In the case of the dilution experiments the photometric precision of the diluted dye

measurement (SD averaging 0,00116 Absorbance units) was less than one third of the smallest overall SD measured (0,00410 for the 20 µl SMI Micro/Pettor.

In these latter experiments an additional source of variability was the volumes of water into which the microvolumes of dyed serum were pipetted. Working here in terms of CV, the average figure for the Selectapette when dispensing 1,0 ml of water (0,13%) was under one fifth of the average CV for the 10 µl Micro/Pettor. Water volume measurements for the experiments on the 20 µl SMI and for the Oxford Dilutor also had CVs less than half the overall values.

Consequently, the figures for volumetric precision are not greatly augumented by secondary sources of variability.

5.7.2.3 Volumetric precision findings

With the 10 ml capacity Oxford Pipettor there was a general trend towards a lower CV with larger volumes of water dispensed. It is also evident that a better precision in the dispensing of one millilitre of water is obtained when using the 1,0 ml capacity Pipettor at the top of its range than when using the 10 ml capacity Pipettor at the bottom of its range. For this volume the best performance was given by the Selectapette, but for the delivery of 0,5 ml there was no difference between the smaller Oxford Pipettor and the Selectapette. Contrary to expectation, the 10 µl SMI Micro/Pettor performed marginally better than the 20 µl model.

In respect of working time, the semiautomated devices operated

about three times as fast as the mouth-operated pipettes, with an equivalent degree of precision for 2,0 ml and an improved precision for 0,5 ml volumes.

5.7.3 Sample carry-over

With both SMI Micro/Pettors the degree of carry-over (under 0,1%) was negligible. In contrast, the carry-over with the plastic tip for the Oxford 20 µl sampler was around fifty times higher, although still unlikely to lead to serious analytical error (a carry-over of 2,4% would cause a serum urea level of 5,0 mmol/l sampled immediately after a specimen of 25 mmol/l to read 5,5 mmol/l). At the cost of an increased sampling time, carry-over can be avoided by using a fresh plastic tip for each sample.

5.7.4 Photometric precision and drift

In the absorbance measurements on inorganic grey solution the Gilford demonstrated a somewhat better precision, compared with the Leitz colorimeter, for readings made in a quarter of the time.

The photometric precision of readings of the final coloured products (Table XIV) did not exceed a CV of 0,5% for any estimation apart from albumin, where the mean absorbance measured was lowest.

Fading of the final colours, giving rise to downward drifts of up to 0,23% per minute, was observed with all the tests other than creatinine, where colour development was evidently still taking place beyond the initial 15 minute colour development time.

Since the largest batch-size (10 tests) used in the later portions of this work could be read on the Gilford in under two

minutes, this upward drift, partly compensated by the time-sequence of adding the alkaline picrate reagent, would cause a maximum error not exceeding 1%, or 1 µmol/l in the normal range.

Carry-over of the final coloured solution in the Gilford flow-cell did not exceed 1% under the standardised sampling conditions employed, with a sampling time of 0,3 seconds causing an aspiration volume of approximately 0,8 ml.

5.7.5 Within-batch precision and correlation of standards

With the exception of the Harleco gasometric procedure for bicarbonate, all the within-batch CVs were under 1,5%. An odd and unexplained finding was that the CV for glucose estimation was noticeably lower than that for cholesterol, based on a similar technique, and was in fact lower than the volumetric precision of the method as determined by the addition of 20 µl of dyed serum to 2,0 ml of water.

The values for the serum-based standards, when read after calibration with the aqueous standards, agreed quite closely with their "set point" values for the SMA 6/60 and 12/60 except for glucose and cholesterol. The Technicon methods for which these standards are primarily intended are the neocuproine method for glucose and a modified Liebermann-Burchard procedure for cholesterol, and although "enzymatic" values for the standards are provided, as shown in Table XVI, exact details of the methods concerned are not stated.

5.7.6 Analyses on patients' specimens

5.7.6.1 Interpretation of between-batch precision data

The between-batch standard deviation of the pairs of duplicate results shown in Table XVII (a - o) was obtained by the expression:[100]

$$SD = \sqrt{\frac{\Sigma d^2}{2N}}$$

where d is the difference between the paired results and N is the number of pairs.

In such an approach, the existence of any overall "bias" of one set of duplicates relative to the other (indicated by a difference in the overall means of the two sets) will contribute to the measured variability, as illustrated in the four sets of figures shown below.

Set	A	B	C	D
	100	103	104	105
	99	97	98	99
	101	102	103	104
	100	98	99	100
	99	101	102	103
	101	99	100	101
	98	100	101	102
	102	101	102	103
	97	99	100	101
	103	100	101	102
Mean	100	100	101	102
Σd^2		44	54	84
$\sqrt{\frac{\Sigma d^2}{2N}}$		1,483	1,643	2,049

Set A represents the first of the duplicate results on a series of ten specimens, while sets B, C and D represent possible values for the second duplicate result. For set B there is no overall bias, while sets C and D carry an overall bias of one and two units respectively. The values for the difference d are relative to the values for A in each of the three sets.

A bias of this nature can result from the fact that the results for each run of assays are read from one single standard, rather than from the mean of a set of standards.

A bias of magnitude b will increase the value for Σd^2 by Nb^2, so that the calculated SD will now be

$$\sqrt{\frac{\Sigma d^2 + Nb^2}{2N}}$$

The effect of bias can be corrected for as follows:-

1) Square the calculated SD to give

$$\frac{\Sigma d^2 + Nb^2}{2N}$$

2) Subtract $\frac{Nb^2}{2N}$ $(= 0,5b^2)$

3) Take the square root to give the corrected SD.

For example, in Set D, the corrected SD is given by

$$\sqrt{(2,049)^2 - 0,5(2^2)} = 1,483$$

If the bias is equal to the initially calculated SD, the

corrected SD is given by

$$\sqrt{SD^2 - 0,5(SD^2)} = 0,71\ SD,$$

while if the bias is equal to half of the initially calculated SD the corrected SD will equal 0,935 SD.

The results presented in Table XVIII indicate that differences in the means of the first and second duplicates occurred to some extent in all cases, associated with imbalances between the numbers of positive and negative values for (b-a). However, apart from the duplicate urea determinations by the diacetyl method, these differences are of low statistical significance as assessed by the paired "t" test (limiting value of t for 19 degrees of freedom = 2,09 for probability P = 0,05). The maximum reduction in calculated SD value as a result of correction, which occurs with the above urea results, is equal to 12%:

$$\frac{0,297 - 0,261}{0,297} \times 100\%.$$

5.7.6.2 Correlation and regression data

Where all the values for x and y in a correlation study are closely grouped, a given degree of variability between individual x and y results can give markedly different values for the regression equation constants and the correlation coefficient, than where the range of values is more extended. This is illustrated in the following sets of figures:-

	Set A		Set B	
	x	y	x	y
	98	99	20	21
	99	98	60	59
	100	101	100	101
	100	99	100	99
	101	102	140	141
	102	101	180	179
Mean	100	100	100	100

Regression equation: $y = 0,80x + 20$ $y = 0,995x + 0,5$

$r = 0,7303$ $r = 0,9998$

In both sets of results the "y" figures were obtained by successively adding and subtracting one from the corresponding "x" figures.

A subjective assessment, based on scrutiny of the figures, would be more useful than the correlation coefficient in the case of set A, when deciding on their degree of correlation.

In the results presented in Table XVII the two poorest correlation coefficients are found in those tests showing a narrow range of values (sodium and calcium), whereas the two best correlation coefficients are found in those tests (urea and glucose) showing a wide range of values.

5.7.6.3 Between-batch precision and correlation findings

The between-batch precision figures shown in Table XVIII can be tabulated alongside the corresponding figures for photometric precision and within-batch precision of standards for several of

the assays, as shown below.

Test	Mean photometric CV %	Mean within-batch CV %	Mean between-batch CV %
Sodium	-	0,32	0,56
Potassium	-	0,64	1,59
Chloride	-	0,42	1,04
Bicarbonate	-	4,6	2,33
Urea	0,36	1,13	2,62
Creatinine	0,43	1,10	2,97
Glucose	0,13	0,19	2,88
Albumin	1,26	1,49	1,78
Calcium	0,36	1,26	2,08
Cholesterol	0,19	0,90	2,44

These figures confirm the findings of other workers[88,101] that the variability associated with photometric absorbance measurement makes a relatively minor contribution to the overall variability, which is due principally to volumetric and chemical factors. Furthermore, the variability encountered "between-batch" is higher than that within-batch in all cases apart from bicarbonate. In the case of the latter (but also with chloride) the distinction between "within-batch" and "between-batch" is somewhat artificial, since every separate assay can be regarded as "between occasion".

In the case of urea, glucose, alkaline phosphatase and aspartate transaminase, the precision was calculated for both the complete set of 20 specimens and also for a subgroup comprising those with values in the "normal range". Values for the standard deviation are closely similar in the two sets for all four assays, but the higher mean value for the complete set causes a noticeable

reduction in the CV.

Of the two supplementary assay procedures investigated, the urease/Berthelot urea method showed a somewhat improved precision, compared with the diacetyl monoxime procedure, at a certain sacrifice in technical simplicity. The modified Liebermann-Burchard cholesterol method proved inferior to the enzymatic one and gave values, on average, around 7% higher.

Examination of the comparable results (excluding the two enzyme assays) by the manual and automated methods shows differences in mean values occurring in all cases, these being particularly noticeable for potassium, chloride, bicarbonate and calcium. Since the specimens had all been analysed during the same daily run on the SMA 6/60 or 12/60 (but on separate occasions for the twelve different tests), these differences could result from influences operating "on the day" in respect of the automated systems. An assessment of the significance of these differences was therefore deferred until data had been obtained (Chapter 6) on a larger number of parallel assays performed in groups over five separate days. The opportunity to perform such a correlative study arose during the assessment of the "reference range" values for a group of 40 subjects, as described in the following chapter.

CHAPTER 6

6. REFERENCE VALUES FOR BLOOD DONORS BY THE MANUAL AND AUTOMATED METHODS

6.1 Protocol for the survey

Single assays for each of the twelve analytes covered in this work, plus four additional analytes (total protein, inorganic phosphorus, uric acid and bilirubin) selected for later inclusion in the SAIMR Interlaboratory Quality Control Programme, were performed by the standard manual methods and also by the SMA 6/60 and 12/60 analysers. In the following presentation of results and discussion only the foregoing twelve assays are included.

The subjects for the survey were forty blood donors attending the Blood Transfusion Centre in Johannesburg, and comprised groups of twenty males (mean age 37 years, range 20 - 65 years) and twenty females (mean age 32 years, range 19 - 62 years). From each subject two 10 ml glass Venoject tubes of blood were collected by the staff of the Centre.

Five batches of eight blood specimens (four male and four female in each batch) were obtained from donors attending between 9.00 a.m. and 11.30 a.m. on the following days:-

Monday	27/8/79
Thursday	30/8/79
Monday	3/9/79
Thursday	6/9/79
Monday	10/9/79

The sets of specimens were collected from the Blood

Transfusion Centre at noon on each day and transported to the SAIMR. Separation of serum after centrifugation was carried out immediately on those samples where clot retraction had proceeded sufficiently to permit this; with the more recently collected specimens a period of standing of up to one hour was necessary before serum separation. The serum was stored at $4^{o}C$ prior to analysis.

The following time-schedule was adopted for the performance of the analyses:-

Day 1 (Afternoon of day of collection):

Sodium, potassium, glucose, aspartate transaminase, alkaline phosphatase. Performance of 6/60 and 12/60 profiles at the Johannesburg General Hospital Laboratory.

Day 2 Chloride, urea, creatinine, calcium, cholesterol, albumin.

Day 3 Bicarbonate.

Collection and recording of all results.

The basis for the above arrangements was the geographical situation of some of the items of equipment (the IL Flame Photometer being in the same laboratory as the automated analysers), and the desire to perform the glucose and enzyme determinations with a minimum of delay.

In order to monitor the accuracy of the assays, two Wellcome "Assayed" control sera were analysed along with the survey specimens. These sera were surplus material from the Wellcome

Quality Control Programme, serum "M2" having originally been issued on 23/5/77 and serum "O2" on 20/6/77. For the manual assays the two sera were analysed once prior to the start of the survey and subsequently in each of the five batches of specimens, providing six between-batch results in all. The same two sera were put up in the first, third and fifth sets of analyses on the 6/60 and 12/60 analysers.

6.2 Results

The individual results for all assays are presented in Table XIX. Table XX provides a breakdown of these results in the form of the mean, standard deviation, observed range and "calculated" reference range (mean ± 2 SD) for the male, female and combined groups respectively, together with the reference ranges currently quoted in the Handbook of the Department of Medical Biochemistry[11] for analyses performed on the 6/60 and 12/60 analysers. Table XXI shows the manual and automated results for the two Wellcome control sera, together with the method means obtained by participants in the Wellcome Programme.

6.3 Discussion

6.3.1 Distribution of results

The reference ranges quoted in the Departmental Handbook for the Technicon analysers had originally been obtained in 1976 by a survey on 200 blood donors. The 6/60 and 12/60 ranges obtained in the present survey showed close agreement with the earlier findings in the case of sodium, potassium, chloride, bicarbonate,

TABLE XIX. Reference value survey results : Males

No.	Age	SODIUM (mmol/l)		POTASSIUM (mmol/l)		CHLORIDE (mmol/l)	
		6/60	Manual	6/60	Manual	6/60	Manual
1	23	140	139	4,3	4,2	99	101
2	58	142	139	5,0	4,6	100	100
3	65	143	139	4,1	3,9	100	99
4	41	139	141	4,1	4,0	100	103
5	28	142	142	4,0	4,0	100	103
6	32	142	143	4,0	4,1	102	103
7	22	140	137	4,7	4,6	102	102
8	58	135	140	4,9	6,1	102	104
9	43	135	141	4,6	4,9	101	103
10	30	141	141	4,6	4,7	102	104
11	32	143	142	4,5	4,6	106	105
12	25	144	142	4,3	4,5	102	102
13	42	144	142	4,8	4,6	105	106
14	37	142	141	4,6	4,6	104	103
15	22	139	141	4,0	4,2	105	103
16	61	142	143	4,3	4,5	105	103
17	20	140	140	4,6	4,4	102	100
18	32	145	144	4,5	4,5	106	105
19	27	142	140	4,2	4,2	101	101
20	40	142	139	4,8	4,8	102	102
Mean	37	141,100	140,800	4,445	4,450	102,450	102,600
Range	20-65	135-145	137-144	4,0 - 5,0	3,9 - 5,1	99-106	99-106
SD		2,634	1,705	0,315	0,320	2,089	1,789

TABLE XIX continued : Males

No.	BICARBONATE (mmol/l)		UREA (mmol/l)		CREATININE (µmol/l)	
	6/60	Manual	6/60	Manual	6/60	Manual
1	22	21	4,1	3,6	91	101
2	26	25	6,3	6,1	99	101
3	23	21	4,6	4,2	90	95
4	24	22	7,7	8,0	125	119
5	20	22	4,2	4,6	95	96
6	23	25	5,7	5,8	95	101
7	18	22	6,3	6,3	95	88
8	25	25	5,9	6,1	88	82
9	22	20	5,2	5,3	109	100
10	22	23	4,5	4,4	89	97
11	20	25	4,2	4,3	95	92
12	28	29	4,5	4,8	109	104
13	24	28	7,2	7,2	109	103
14	23	26	6,9	6,9	97	94
15	24	26	4,6	4,5	96	88
16	26	29	9,1	8,9	102	90
17	25	27	4,0	3,3	96	84
18	27	26	5,8	6,1	109	94
19	25	26	3,4	3,8	96	94
20	25	24	6,4	6,7	96	110
Mean	23,60	24,60	5,530	5,545	99,050	96,650
Range	18-28	20-29	3,4 - 9,1	3,3 - 8,9	88-125	82-119
SD	2,479	2,664	1,464	1,520	9,047	8,750

TABLE XIX continued : Males

	GLUCOSE (mmol/l)		ALBUMIN (g/l)		CALCIUM (mmol/l)	
No.	12/60	Manual	12/60	Manual	12/60	Manual
1	4,6	4,4	46	42	2,25	2,31
2	4,8	4,0	43	38	2,31	2,30
3	4,5	4,4	51	48	2,35	2,35
4	4,7	4,3	44	43	2,25	2,34
5	3,6	3,3	48	48	2,36	2,42
6	4,6	4,1	45	44	2,27	2,32
7	5,1	4,8	47	46	2,27	2,40
8	4,0	3,5	43	42	2,21	2,33
9	6,2	5,8	44	42	2,25	2,44
10	4,8	4,2	49	46	2,41	2,55
11	5,6	4,9	40	39	2,34	2,40
12	4,8	4,6	49	47	2,22	2,35
13	5,9	5,8	44	42	2,19	2,32
14	5,2	4,8	46	46	2,31	2,35
15	4,8	4,7	45	45	2,26	2,38
16	4,8	4,5	43	41	2,19	2,28
17	4,8	3,9	50	48	2,28	2,40
18	5,2	4,7	45	42	2,21	2,38
19	3,9	3,5	45	46	2,33	2,37
20	4,0	3,7	48	49	2,17	2,31
Mean	4,795	4,395	45,750	44,200	2,272	2,365
Range	3,6 - 6,2	3,3 - 5,8	40-51	38-49	2,19 - 2,41	2,28 - 2,55
SD	0,646	0,672	2,770	3,156	0,0647	0,0612

TABLE XIX continued : Males

No.	CHOLESTEROL (mmol/l)		ALKALINE PHOSPHATASE (IU/l)		ASPARTATE TRANSAMINASE (IU/l)	
	12/60	Manual	12/60	Manual	12/60	Manual
1	4,38	4,56	77	29,2	24	15
2	5,11	5,51	57	22,3	27	19
3	6,12	6,33	91	32,8	16	15
4	6,18	6,30	64	23,9	14	19
5	8,53	8,40	68	25,0	31	25
6	4,86	5,00	50	18,8	18	16
7	6,93	6,93	101	36,5	34	20
8	5,38	5,46	56	21,8	18	12
9	5,56	5,69	85	32,7	19	12
10	5,25	5,19	73	27,6	21	14
11	4,27	4,41	69	27,4	22	21
12	5,41	5,72	37	13,1	18	15
13	6,25	6,31	75	27,0	17	14
14	5,97	6,49	47	15,1	15	12
15	4,58	4,76	55	23,1	20	15
16	5,96	6,22	57	21,7	30	27
17	5,55	5,71	98	37,8	18	17
18	5,48	5,76	49	19,3	18	14
19	4,97	5,17	63	21,1	16	13
20	4,51	4,67	66	22,6	29	25
Mean	5,563	5,730	66,900	24,940	21,250	17,000
Range	4,27 - 8,53	4,41 - 8,40	37-101	13,1 - 37,8	14-34	12-27
SD	0,987	0,942	17,207	6,550	5,893	4,565

TABLE XIX continued : Females

No.	Age	SODIUM (mmol/l)		POTASSIUM (mmol/l)		CHLORIDE (mmol/l)	
		6/60	Manual	6/60	Manual	6/60	Manual
1	39	139	138	4,4	4,5	102	100
2	21	146	146	4,3	4,2	97	97
3	35	141	140	3,9	3,8	103	101
4	20	140	139	4,2	4,1	106	104
5	43	137	141	4,1	4,2	99	103
6	30	140	142	3,8	3,8	104	104
7	47	142	140	4,2	4,0	102	100
8	20	143	144	4,1	4,0	103	105
9	20	140	139	4,0	4,3	104	106
10	24	137	136	4,1	4,2	99	100
11	21	142	139	4,8	4,6	102	102
12	19	140	142	4,0	4,2	106	107
13	35	143	143	4,1	4,1	105	104
14	53	142	141	4,3	4,2	103	101
15	52	147	144	4,6	4,4	105	104
16	37	140	140	4,1	4,1	105	103
17	28	140	140	4,4	4,3	105	103
18	18	143	144	4,4	4,7	106	104
19	19	141	140	4,6	4,4	104	105
20	62	143	142	4,8	4,8	106	106
Mean	32	141,000	141,000	4,260	4,245	103,300	102,950
Range	19-62	137-147	136-146	3,8 - 4,8	3,8 - 4,8	97-106	97-107
SD		2,515	2,406	0,280	0,267	2,557	2,502

TABLE XIX continued : Females

No.	BICARBONATE (mmol/l)		UREA (mmol/l)		CREATININE (µmol/l)	
	6/60	Manual	6/60	Manual	6/60	Manual
1	24	25	6,8	6,5	77	82
2	16	17	5,1	4,5	81	96
3	25	28	5,6	5,8	60	70
4	20	21	5,1	5,3	78	78
5	22	18	3,7	3,8	70	82
6	23	21	3,0	3,3	55	70
7	23	20	2,4	2,6	77	82
8	26	23	2,6	2,7	71	77
9	20	23	3,6	3,1	84	77
10	19	21	5,2	5,0	75	72
11	21	25	2,1	1,7	49	59
12	21	24	4,0	3,8	55	63
13	24	29	8,1	8,5	124	112
14	24	27	7,2	4,9	107	99
15	23	25	9,3	9,1	119	110
16	24	27	4,1	3,2	66	63
17	24	27	3,6	3,5	82	74
18	26	30	6,6	6,9	82	80
19	24	28	3,9	3,8	71	79
20	21	25	7,5	7,3	72	79
Mean	22,500	24,200	4,975	4,765	77,750	80,200
Range	16-26	17-30	2,1 - 9,3	1,7 - 9,1	49-124	59-112
SD	2,503	3,651	2,039	2,031	19,588	14,318

TABLE XIX continued : Females

No.	GLUCOSE (mmol/l)		ALBUMIN (g/l)		CALCIUM (mmol/l)	
	12/60	Manual	12/60	Manual	12/60	Manual
1	4,2	4,4	42	42	2,41	2,44
2	8,7	7,8	42	39	2,26	2,24
3	4,4	4,5	43	43	2,15	2,22
4	4,4	4,3	46	47	2,32	2,38
5	4,2	4,1	47	47	2,28	2,31
6	6,1	5,8	47	46	2,22	2,25
7	4,9	4,6	44	42	2,28	2,38
8	4,5	4,5	44	44	2,22	2,30
9	4,4	4,2	39	38	2,22	2,35
10	5,2	4,5	47	44	2,32	2,51
11	4,7	4,2	45	42	2,32	2,45
12	4,7	4,3	45	45	2,27	2,46
13	5,2	4,9	43	42	2,25	2,32
14	4,3	3,7	44	42	2,28	2,35
15	4,7	3,9	44	41	2,45	2,44
16	4,5	3,8	45	42	2,22	2,34
17	4,3	3,9	43	41	2,15	2,24
18	6,0	5,7	47	48	2,31	2,45
19	4,9	4,7	46	46	2,26	2,40
20	3,5	3,3	42	43	2,31	2,43
Mean	4,890	4,555	44,250	43,200	2,275	2,364
Range	3,5 - 8,7	3,3 - 7,8	39-47	38-48	2,15 - 2,45	2,22 - 2,51
SD	1,079	0,970	2,124	2,668	0,0732	0,0859

TABLE XIX continued : Females

No.	CHOLESTEROL (mmol/l)		ALKALINE PHOSPHATASE (IU/l)		ASPARTATE TRANSAMINASE (IU/l)	
	12/60	Manual	12/60	Manual	12/60	Manual
1	5,94	6,31	46	16,9	24	17
2	3,97	4,08	42	14,0	18	19
3	3,53	3,81	61	21,3	23	18
4	3,66	3,93	51	17,6	13	15
5	6,56	6,50	53	19,5	20	13
6	4,17	4,30	33	14,6	9	12
7	5,75	5,50	57	19,7	9	17
8	3,65	3,80	47	18,0	21	16
9	4,45	4,46	43	15,1	24	16
10	4,49	4,47	81	26,7	26	16
11	4,36	4,43	80	29,8	23	26
12	3,24	3,34	75	27,6	26	28
13	5,39	5,23	79	23,2	20	17
14	3,35	3,50	68	23,3	20	14
15	5,49	5,18	79	29,8	18	12
16	5,04	4,86	72	23,5	22	19
17	4,42	4,51	57	20,4	15	19
18	5,13	5,26	54	20,0	29	14
19	4,26	4,46	36	13,5	16	26
20	5,04	5,37	72	23,8	22	28
Mean	4,595	4,665	59,300	20,915	19,900	18,100
Range	3,24 - 6,56	3,34 - 6,50	33-81	13,5 - 29,8	9-29	12-28
SD	0,924	0,860	15,557	5,020	5,389	5,046

TABLE XX. Reference value ranges

Assay	Males	Females	Overall
SODIUM (mmol/l)			
1.L			
Actual range	137 - 144	136 - 146	136 - 146
Mean	140,800	141,000	140,900
SD	1,705	2,406	2,061
Derived range	137 - 144	136 - 146	137 - 145
Sex difference of means	N/S		
6/60			
Actual range	135 - 145	137 - 147	135 - 147
Mean	141,100	141,300	141,200
SD	2,634	2,515	2,544
Derived range	136 - 146	136 - 146	136 - 146
Sex difference of means	N/S		
Handbook range			135 - 148
POTASSIUM (mmol/l)			
1.L			
Actual range	3,9 - 5,1	3,8 - 4,8	3,8 - 5,1
Mean	4,450	4,245	4,348
SD	0,320	0,267	0,309
Derived range	3,8 - 5,1	3,7 - 4,8	3,7 - 5,0
Sex difference of means	t = 2,2		
6/60			
Actual range	4,0 - 5,0	3,8 - 4,8	3,8 - 5,0
Mean	4,445	4,260	4,353
SD	0,315	0,280	0,309
Derived range	3,8 - 5,1	3,7 - 4,8	3,7 - 5,0
Sex difference of means	N/S		
Handbook range			3,8 - 5,2

TABLE XX continued

Assay	Males	Females	Overall
CHLORIDE (mmol/l)			
Chloride meter			
Actual range	99 - 106	97 - 107	97 - 107
Mean	102,600	102,950	102,775
SD	1,789	2,502	2,154
Derived range	99 - 106	98 - 108	98 - 107
Sex difference of means	N/S		
6/60			
Actual range	99 - 106	97 - 106	97 - 106
Mean	102,45	103,300	102,875
SD	2,089	2,557	2,345
Derived range	98 - 107	98 - 108	98 - 108
Sex difference of means	N/S		
Handbook range			98 - 109
BICARBONATE (mmol/l)			
Harleco			
Actual range	20 - 29	17 - 30	17 - 30
Mean	24,600	24,200	24,40
SD	2,664	3,651	3,16
Derived range	19 - 30	17 - 32	18 - 31
Sex difference of means	N/S		
6/60			
Actual range	18 - 28	16 - 26	16 - 28
Mean	23,600	22,500	23,05
SD	2,479	2,503	2,52
Derived range	19 - 29	17 - 28	18 - 28
Sex difference of means	N/S		
Handbook range			18 - 28

TABLE XX continued

Assay	Males	Females	Overall
UREA (mmol/l)			
Manual			
Actual range	3,3 - 8,9	1,7 - 9,1	1,7 - 9,1
Mean	5,545	4,765	5,155
SD	1,520	2,031	1,814
Derived range	2,5 - 8,6	0,7 - 8,3	1,5 - 8,8
Sex difference of means	N/S		
6/60			
Actual range	3,4 - 9,1	2,1 - 9,3	2,1 - 9,3
Mean	5,530	4,975	5,253
SD	1,464	2,039	1,774
Derived range	2,6 - 8,5	0,9 - 9,1	1,7 - 8,8
Sex difference of means	N/S		
Handbook range			2,5 - 6,6
CREATININE (µmol/l)			
Manual			
Actual range	82 - 119	59 - 112	59 - 119
Mean	96,650	80,200	88,425
SD	8,750	14,318	14,372
Derived range	79 - 114	52 -109	60 - 117
Sex difference of means	t = 4,384		
6/60			
Actual range	88 - 125	49 - 124	49 - 125
Mean	99,050	77,750	88,400
SD	9,047	15,588	18,523
Derived range	81 - 117	39 - 117	51 - 125
Sex difference of means	t = 4,414		
Handbook range			60 - 120

TABLE XX continued

Assay	Males	Females	Overall
GLUCOSE (mmol/l)			
Manual			
Actual range	3,3 - 5,8	3,3 - 7,8	3,3 - 7,8
Mean	4,395	4,555	4,475
SD	0,672	0,970	0,828
Derived range	3,1 - 5,7	2,6 - 6,5	2,8 - 6,1
Sex difference of means	N/S		
12/60			
Actual range	3,6 - 6,2	3,5 - 8,7	3,5 - 8,7
Mean	4,795	4,890	4,843
SD	0,646	1,079	0,879
Derived range	3,5 - 6,1	2,7 - 7,0	3,1 - 6,6
Sex difference of means	N/S		
Handbook range			3,4 - 6,1
ALBUMIN (g/l)			
Manual			
Actual range	38 - 49	38 - 48	38 - 49
Mean	44,200	43,200	43,700
SD	3,156	2,668	2,298
Derived range	38 - 51	38 - 49	38 - 50
Sex difference of means	N/S		
12/60			
Actual range	40 - 51	39 - 47	39 - 51
Mean	45,750	44,250	45,000
SD	2,770	2,124	2,552
Derived range	40 - 51	40 - 48	40 - 50
Sex difference of means	N/S		
Handbook range			30 - 55

TABLE XX continued

Assay	Males	Females	Overall
CALCIUM (mmol/l)			
Manual			
Actual range	2,28 - 2,55	2,22 - 2,51	2,22 - 2,55
Mean	2,365	2,364	2,364
SD	0,0612	0,0859	0,0736
Derived range	2,24 - 2,49	2,19 - 2,54	2,22 - 2,51
Sex difference of means	N/S		
12/60			
Actual range	2,19 - 2,41	2,15 - 2,45	2,15 - 2,45
Mean	2,272	2,275	2,273
SD	0,0647	0,0732	0,0683
Derived range	2,14 - 2,40	2,13 - 2,42	2,14 - 2,41
Sex difference of means	N/S		
Handbook range			2,15 - 2,70
CHOLESTEROL (mmol/l)			
Manual			
Actual range	4,41 - 8,40	3,34 - 6,50	3,34 - 8,40
Mean	5,370	4,665	5,198
SD	0,942	0,860	1,041
Derived range	3,85 - 7,61	2,95 - 6,39	3,12 - 7,28
Sex difference of means	t = 3,716		
12/60			
Actual range	4,27 - 8,53	3,24 - 6,56	3,24 - 8,53
Mean	5,563	4,595	5,079
SD	0,987	0,924	1,063
Derived range	3,59 - 7,54	2,75 - 6,44	2,95 - 7,19
Sex difference of means	t = 3,202		
Handbook range			3,12 - 6,76

TABLE XX continued

Assay	Male	Female	Overall
ALKALINE PHOSPHATASE (U/l)			
Manual			
Actual range	13,1 - 37,8	13,5 - 29,8	13,1 - 37,8
Mean	24,940	20,915	22,928
SD	6,500	5,020	6,110
Derived range	12 - 38	11 - 31	11 - 35
Manufacturer's range			9 - 35
Sex difference of means	t = 2,181		
12/60			
Actual range	37 - 101	33 - 81	33 - 101
Mean	66,900	59,300	63,100
SD	17,207	15,557	16,642
Derived range	32 - 101	28 - 90	30 - 96
Sex difference of means	N/S		
Handbook range			30 - 100
ASPARTATE TRANSAMINASE (U/l)			
Manual			
Actual range	12 - 27	12 - 28	12 - 28
Mean	17,000	18,000	17,000
SD	4,565	5,046	4,782
Derived range	8 - 26	8 - 28	8 - 27
Manufacturer's range	7 - 26	6 - 25	
Sex difference of means	N/S		
12/60			
Actual range	14 - 34	9 - 29	9 - 34
Mean	21,250	19,900	20,575
SD	5,893	5,389	5,615
Derived range	9 - 33	9 - 31	9 - 32
Sex difference of means	N/S		
Handbook range (for 12/60)			5 - 40

TABLE XXI. Analyses of Wellcome "Assayed" control sera during reference value survey

Test	Specimen	MANUAL				6/68 or 12/68	
		Mean	SD	CV %	Method Mean	Rounded Mean	Method Mean
Sodium	M2	128,2	0,41	0,32	128	129	128
	02	138,3	0,52	0,37	138	140	139
Potassium	M2	3,55	0,055	1,54	3,57	3,6	3,57
	02	4,02	0,041	1,02	4,02	4,0	4,04
Chloride	M2	88,7	0,52	0,58	88,8	89	89,7
	02	96,2	0,75	0,78	96,6	94	97,5
Bicarbonate	M2	19,3	2,10	10,9	18,7	18,0	18,5
	02	20,0	1,55	7,7	19,1	18,6	19,5
Urea	M2	7,42	0,55	7,4	7,5	7,5	7,42
	02	17,74	0,16	0,9	17,4	17,8	17,9
Creatinine	M2	185,5	2,43	1,31	182	185	181
	02	238,2	7,76	3,26	245	255	242
Glucose	M2	4,63	0,18	3,9	4,61	4,74	4,89
	02	7,36	0,19	2,6	7,32	7,14	7,65
Albumin	M2	35,08	3,10	8,9	34,4	35,2	35,0
	02	36,82	2,34	6,4	38,1	38,6	38,6
Calcium	M2	2,53	0,062	2,4	2,53	2,45	2,45
	02	2,99	0,067	2,2	2,93	2,89	2,90
Cholesterol	M2	3,46	0,096	2,8	3,58	3,53	3,50
	02	2,64	0,082	3,1	2,80	2,57	2,71
Alkaline phosphatase	M2	51,95	2,54	4,9	60	164	156
	02	38,55	2,06	5,3	45	118	109
Aspartate transaminase	M2	42,23	3,41	8,1	44,2 *	65	(Not given)
	02	37,08	1,57	4,2	37,7 *	55	(Not given)

*Stated method means at 37°C converted to 30°C using temperature factor given by Worthington (6,70)

creatinine and alkaline phosphatase. For albumin, calcium and aspartate transaminase the present ranges were narrower, while for urea, glucose and cholesterol they were wider.

In the case of glucose and cholesterol the extension of the range can be attributed to the influence, in the relatively small survey population, of single high "outliers". The glucose value of 8,7 mmol/l in female subject (2) is 2,5 mmol/l higher than the next highest value of 6,2 mmol/l in male subject (9), while the cholesterol value of 8,53 mmol/l in male subject (5) is 1,6 mmol/l higher than the next highest value of 6,92 in male subject (7).

Trimming of the results by exclusion of the highest and lowest of the forty values, to give the 95 percentile limits, gives ranges in much closer agreement with the earlier figures (glucose 3,58 - 5,97 mmol/l; cholesterol 3,27 - 6,80 mmol/l).

In the case of urea the limits of the Handbook range were adjusted to conform with the long accepted range of "15 - 40 mg/100 ml". In the present survey nine subjects (4 male and 5 female) had urea values above this upper limit of 6,6 mmol/l. There is closer agreement to an upper limit of 8,3 mmol/l proposed by Varley, Gowenlock and Bell,[102] only two of the nine values exceeding the latter figure. The non-Gaussian distribution resulting from the existence of the outlying results for cholesterol and glucose makes it inappropriate to apply the statistical tests of significance (t test and F test) for these two analytes.

6.3.2 Sex differences of means

With the exclusions noted above, the significance of differences between the means for the male and female groups was assessed by the t test.[100] For 38 degrees of freedom (20 + 20 - 2) the critical value for t (P = 0,05) is 2,02. At this level, a significant difference in mean (M > F) was found for creatinine by both the manual and automated methods. In the case of potassium and alkaline phosphatase the higher mean levels for males were significant only for the manual methods; in both cases the t value was only marginally above the critical value.

The higher male values for creatinine, together with those evident but not statistically tested for cholesterol, accord with accepted findings for these two assays.[102]

6.3.3 Sex differences of ranges

The significance of differences in the standard deviations of the male and female groups was assessed by the F test.[100] For 19 degrees of freedom in the numerator and denominator the critical value for F (P = 0,05) is 2,17. At this level a significant difference occurred with creatinine, the standard deviation for females being higher than that for males of both the manual method (F = 2,68) and on the SMA 6/60 (F = 4,69).

6.3.4 Analytical differences of means

The significance of differences in the overall mean values for all forty subjects when analysed by the manual and automated procedures was assessed by the paired t test.[100] For 19 degrees of freedom

(20 - 1) the critical value for t (P = 0,05) is 2,09. At this level the differences in means were not significant for sodium, potassium, chloride, urea and creatinine, but significant differences were encountered with three of the remaining comparable results, as indicated below.

Analyte	Mean manual result (M)	Mean automated result (A)	(M-A)	t
Bicarbonate (mmol/l)	24,40	23,05	1,35	3,52
Albumin (g/l)	43,70	45,00	-1,30	5,40
Calcium (mmol/l)	2,364	2,273	0,091	10,31

The nature and extent of these differences diverge in several instances from those found on a "single day" basis (Table XVII). For bicarbonate, calcium and albumin the relative order of the means is reversed, while for creatinine, where previously a difference of around 7% existed, the method means are now practically identical.

The validity of these method-differences on the survey subjects is substantiated by examination of the corresponding figures for the analysis of the Wellcome control sera (Table XXI). The corresponding average values for [manual result - automated result] are

Bicarbonate	1,35 mmol/l
Albumin	-0,95 g/l
Calcium	0,09 mmol/l

In the case of glucose and cholesterol the previous small differences in method means, indicated in Table XVII, are found to be enlarged in Table XX. For cholesterol the difference is still small (0,119 mmol/l), but for glucose the difference of 0,368 mmol/l

by which the 12/60 mean result exceeds that of the manual method compares with a corresponding level of 0,18 mmol/l (5,01 - 4,83) for the "normal range" values in Table XVII.

The increased magnitude of this method difference is more in accord with the less specific nature of the 12/60 glucose method.

6.3.5 Analytical differences of standard deviations

Application of the F test showed there to be no significant differences in the overall standard deviations for all 40 subjects' results when analysed by the manual and automated methods.

6.3.6 "Accuracy" of the manual methods

If the "method mean" results provided by the participants in the Wellcome Programme are regarded as corresponding to the "most likely" results for those analytes by those particular methods, concordance of the results obtained (Table XXI) provides an indication of analytical accuracy. This can be expressed as the percentage deviation of the mean result from the method mean (taking the average figure for the two control sera), as set out below:

	Manual	Automated
Sodium	+ 0,2%	+ 0,6%
Potassium	- 0,3%	- 0,1%
Chloride	- 0,3%	- 2,5%
Bicarbonate	+ 4,0%	- 4,0%
Urea	+ 1,0%	- 0,2%
Creatinine	- 0,8%	+ 3,6%
Glucose	+ 0,5%	- 5,3%
Albumin	- 0,8%	+ 0,3%
Calcium	+ 1,1%	- 0,2%
Cholesterol	- 4,4%	- 5,0%
Alkaline phosphatase	-13,8%	+ 6,4%
Aspartate transaminase	- 3,2%	-

Examination of the manual results shows there to be a good level of accuracy for those analytes where the small extent of the biological variability renders this most desirable (sodium, chloride, calcium), while potassium, urea, creatinine, glucose and albumin also showed deviations from the method mean not exceeding 1,1%. The most divergent results occurred with the estimation of alkaline phosphatase, possibly accounted for by the fact that the assays were performed within three hours of the reconstitution of the lyophilised sera. Participants in the Wellcome Programme using this method could possibly have allowed, on average, a longer period prior to analysis, with consequent augmenting of the alkaline phosphatase activity of the sera.[103,104] No ready explanation can be put forward for the higher alkaline phosphatase results obtained by the 12/60 relative to the method mean for that instrument.

The 6/60 values for chloride and the 12/60 values for glucose both diverge from their method means to a noticeably greater extent than their manual counterparts.

6.3.7 Between-batch precision of assays on control sera

Compared to the precision data derived from the between-batch analysis of duplicate patients' specimens (Table XVIII), the most noticeable differences when analysing the control sera were the greatly increased CV values for bicarbonate and albumin found with the latter.

These various indices of precision, and their medical

significance, are further discussed in the final chapter of this work.

CHAPTER 7

7. INSTRUMENT AND METHOD PERFORMANCES IN THE SAIMR INTERLABORATORY QUALITY CONTROL PROGRAMME

The SAIMR Quality Control Programme was inaugurated in April 1980 with an initial cycle of four specimens, analysed in each of the months from April to July. Following a pause of one month for assessment of the general operation of the Programme and the early results, a further series of three specimens was issued for analysis in September, October and November 1980.

7.1 Protocol

The 58 participating laboratories included the routine laboratory of the Central Institute and all but two of the 59 Branch Laboratories. Those excluded were the newly-opened laboratory at Middelburg, Transvaal, and the Regional Headquarters laboratory at Windhoek, biochemical work from the latter being taken to the Katatura Hospital laboratory.

The analyses included on the Programme were the twelve featuring in the present work with the addition of total protein, inorganic phosphate, uric acid and total bilirubin. A questionnaire sent out in January 1980 brought in information on which of the sixteen assays each laboratory routinely carried out, together with the instrumentation and methodology being used.

The material for analysis consisted of commercial lyophilised control serum (Technicon "Multi-System Control" and Wellcome Reagents "Wellcomtrol" control serum), as detailed below:

Control No.	Analysis date	Material
1	11/ 4/80	Technicon MSC normal (assayed) Batch X9E 104
2	9/ 5/80	Technicon MSC abnormal (unassayed) Batch X9G 208
3	13/ 6/80	Technicon MSC normal (unassayed) Batch X9F 105
4	11/ 7/80	Technicon MSC abnormal (assayed) Batch X9C 202
5	20/ 9/80	Wellcomtrol One (unassayed) Batch K 9026
6	18/10/80	Wellcomtrol Two (unassayed) Batch K 8913
7	22/11/80	Wellcontrol Three (unassayed) Batch K 7221

The detailed instructions issued to the participating laboratories and the format of the Results Form are shown in Appendices 3 and 4.

7.2 Data processing

As results came in by post from the Branches they were manually transcribed on to a monthly Master Record Sheet; the deadline for receipt of results was set at the Monday ten days after the "set" analysis date. The following operations were then performed, with the aid of a programme written for the Hewlett-Packard HP 97 calculator, for all analytes apart from the two enzymes:-

a) Calculation of the overall mean and standard deviation of all results.

b) Display of the upper and lower limits of the range of mean ± 3 SD.

c) Elimination of results outside these limits and recalculation of the mean and SD for the remaining results.

d) Calculation of the bias of each result, this being expressed as

$$\frac{\text{result - corrected mean}}{\text{corrected SD}}$$

e) Calculation of the mean and standard deviation for the various individual instruments and methods (including the two enzyme assays), only results used for the recalculated overall mean and SD being included.

The bias figures were then manually transcibed on to the original result forms which, after photocopying for record purposes, were returned to the individual senders together with a typed sheet showing the overall and method means and SDs. A summary of the performance of laboratories in each of the eight administrative regions of the SAIMR was sent to the Regional Director, showing all results outside ± 2 SD, and identifying those laboratories which had failed to submit a return for that month.

7.3 Comparison with the United Kingdom National Quality Control Scheme

The structure and operation of the Programme introduced for SAIMR laboratories shows several similarities with the original form of the United Kingdom Scheme, set up in 1969.[105] A number of points of difference are outlined below:

1) Returns are required monthly instead of fortnightly, this being necessitated by the larger geographical area covered and attendant postal delays. (From the analysis date to the date statistical results reach the participants can range

from two to three weeks.)

2) Lyophilised commercial control serum is used in place of Seitz-filtered human serum.

3) The SAIMR Programme covers sixteen analyses on each occasion, whereas the UK Scheme covers fifteen analyses on each pair of occasions (a group of eight assays alternating with a group of seven). Two tests additional to the UK list are included (bicarbonate and aspartate transaminase), while one assay (serum iron) is omitted.

4) A less extensive breakdown into individual methods for the various analytes is attempted with the SAIMR Programme, due to the small numbers of laboratories using some of the less popular procedures.

   A number of the individual methods included in Table XXII were only subjected to statistical analysis at the end of the year and were not reported on each month; among these were the diacetyl monoxime method for urea, reaction-rate methods for creatinine; and the ABA-100 assays for albumin, calcium, cholesterol, alkaline phosphatase and aspartate transaminase.

5) The more sophisticated measure of an individual laboratory's performance, introduced into the UK Scheme after 1970 in the form of the "Variance Index",[105] can only be incorporated into the SAIMR Programme when a wider data base has been accumulated for assigning fixed values to the various co-efficients of variation.

6) Much of the data processing for the SAIMR Programme has

hitherto been done manually, in contrast to the use of the IBM 1130 computer at Birmingham. From March 1981, however, the Programme has been set up on the Institute's Commodore PET minicomputer, using a programme compiled by the Leo Computer Bureau in Johannesburg.

7.4 Results

Only results appertaining to the twelve assays covered in this work are discussed in this and the following sections.

The means, standard deviations and coefficients of variation for various methods for each of the twelve assays on each of the seven control sera are shown in Table XXII (a - m). At the bottom of each column are shown the average figures for the number of analyses, analyte concentration and coefficient of variation for all seven controls, while the right-hand column ("stated values") gives the manufacturer's values for the two "analysed" Technicon Multi-System Controls (specimens 1 and 4).

Standard deviations were not calculated in four cases where only two results for a particular method were available; in these cases the average CV was calculated from six instead of seven values, as indicated in the tables.

Table XXIII shows the number of users of the principal methods, as notified at the beginning of the year, together with the average number of results accepted and the incidence of "wild" results (outside ± 3 SD from the overall mean).

Discrepancy between the total number of users and the total

TABLE XXII. SAIMR Interlaboratory Quality Control results (1980)

(a) SODIUM (mmol/l)

		Overall	6/60	IL	EEL	Stated value
1)	No.	51	6	22	21	
	Mean	140,94	141,33	141,57	140,05	142 (SMA)
	SD	2,63	0,82	1,57	3,58	
	CV %	1,87	0,58	1,11	2,56	
2)	No.	48	6	21	19	
	Mean	148,90	149,00	149,50	147,79	
	SD	3,72	1,26	3,41	4,92	
	CV %	2,50	0,85	2,28	3,33	
3)	No.	53	6	22	23	
	Mean	142,94	143,67	143,48	142,04	
	SD	2,32	0,52	1,48	3,04	
	CV %	1,62	0,36	1,03	2,14	
4)	No.	51	6	21	22	
	Mean	147,52	150,00	149,49	144,94	149 (SMA)
	SD	3,81	2,00	1,69	4,31	
	CV %	2,58	1,33	1,13	2,97	
5)	No.	50	6	21	21	
	Mean	140,33	139,67	140,71	139,99	
	SD	2,31	2,50	1,41	3,09	
	CV %	1,65	1,79	1,00	2,21	
6)	No.	47	6	21	17	
	Mean	147,57	149,17	149,06	145,89	
	SD	3,64	2,23	1,79	4,84	
	CV %	2,47	1,49	1,20	3,32	
7)	No.	54	6	22	23	
	Mean	133,76	133,67	134,33	133,70	
	SD	3,37	1,51	2,35	4,39	
	CV %	2,52	1,13	1,75	3,28	
Mean No.		51	6	21	21	
Mean value		143,14	143,79	144,03	142,06	
Mean CV %		2,17	1,08	1,36	2,83	

TABLE XXII continued

(b) POTASSIUM (mmol/l)

		Overall	6/60	IL	EEL	Stated value
1)	No.	50	6	22	20	
	Mean	4,38	4,34	4,34	4,43	4,4 (SMA)
	SD	0,21	0,10	0,10	0,30	
	CV %	4,8	2,3	2,3	6,8	
2)	No.	48	6	22	18	
	Mean	2,87	2,85	2,88	2,89	
	SD	0,24	0,06	0,31	0,23	
	CV %	8,4	2,1	10,8	8,0	
3)	No.	52	6	22	22	
	Mean	4,46	4,42	4,45	4,48	
	SD	0,09	0,07	0,06	0,12	
	CV %	2,0	1,6	1,3	2,7	
4)	No.	49	6	20	21	
	Mean	3,01	2,97	3,01	3,05	3,0 (SMA)
	SD	0,12	0,07	0,08	0,15	
	CV %	4,0	2,4	2,7	4,9	
5)	No.	49	6	21	20	
	Mean	4,73	4,71	4,76	4,72	
	SD	0,16	0,22	0,06	0,18	
	CV %	3,4	4,7	1,3	3,8	
6)	No.	47	6	21	17	
	Mean	5,29	5,35	5,33	5,26	
	SD	0,24	0,12	0,06	0,33	
	CV %	4,5	2,2	1,1	6,3	
7)	No.	52	6	21	22	
	Mean	4,03	4,07	4,06	4,00	
	SD	0,11	0,10	0,12	0,09	
	CV %	2,7	2,4	3,0	2,3	
Mean No.		50	6	21	20	
Mean value		4,11	4,10	4,12	4,12	
Mean CV %		4,3	2,5	3,2	5,0	

TABLE XXII continued

(c) CHLORIDE (mmol/l)

		Overall	6/60	Radiometer	Schales	Stated value
1)	No.	50	6	25	19	
	Mean	101,23	101,67	100,80	101,64	104 (SMA)
	SD	2,81	1,37	1,98	3,86	
	CV %	2,78	1,35	1,96	3,80	
2)	No.	48	6	25	17	
	Mean	110,24	110,67	109,67	110,88	
	SD	2,86	2,73	1,99	3,88	
	CV %	2,59	2,47	1,81	3,50	
3)	No.	53	6	25	22	
	Mean	102,49	104,33	102,54	102,21	
	SD	2,44	1,97	1,98	3,07	
	CV %	2,38	1,89	1,93	3,00	
4)	No.	49	5	25	19	
	Mean	112,28	111,00	111,83	112,93	115 (SMA)
	SD	2,94	4,74	1,73	3,59	
	CV %	2,62	4,27	1,55	3,18	
5)	No.	47	6	21	20	
	Mean	96,84	96,33	96,64	97,21	
	SD	2,60	3,14	2,06	3,02	
	CV %	2,68	3,26	2,13	3,18	
6)	No.	47	6	24	17	
	Mean	104,86	105,50	104,71	104,85	
	SD	2,58	2,51	2,42	2,92	
	CV %	2,46	2,38	2,31	2,78	
7)	No.	51	6	24	21	
	Mean	92,3	92,00	91,94	93,34	
	SD	3,02	1,10	2,94	3,90	
	CV %	3,27	1,20	3,20	4,18	
Mean No.		49	6	24	19	
Mean value		102,90	103,07	102,57	103,29	
Mean CV %		2,68	2,40	2,13	3,27	

TABLE XXII continued

(d) BICARBONATE (mmol/l)

		Overall	6/60	Harleco	Stated value
1)	No.	38	5	32	
	Mean	5,22	4,26	5,38	4 (SMA)
	SD	1,48	0,83	1,48	
	CV %	28,4	19,5	27,5	
2)	No.	43	5	37	
	Mean	9,88	10,34	9,74	
	SD	2,79	3,58	2,74	
	CV %	28,2	34,6	28,1	
3)	No.	43	5	37	
	Mean	5,81	4,28	5,99	
	SD	1,79	0,44	1,81	
	CV %	30,8	10,3	30,2	
4)	No.	44	5	39	
	Mean	9,36	8,46	9,47	8 (SMA)
	SD	2,17	0,64	2,27	
	CV %	23,2	7,6	24,0	
5)	No.	39	5	33	
	Mean	19,83	18,82	20,11	
	SD	2,49	1,33	2,55	
	CV %	12,6	7,1	12,7	
6)	No.	40	5	34	
	Mean	18,89	18,54	19,02	
	SD	2,84	1,50	3,01	
	CV %	15,0	8,1	15,8	
7)	No.	45	5	39	
	Mean	20,09	20,00	20,14	
	SD	2,47	1,73	2,58	
	CV %	12,3	8,7	12,8	
Mean No.		42	5	36	
Mean value		12,73	12,10	12,84	
Mean CV %		21,5	13,7	21,6	

TABLE XXII continued

(c) UREA (mmol/l)

		Overall	6/60	Beckman	Berthelot	DAM	Stated value
1)	No.	51	6	13	25	4	
	Mean	7,84	7,93	7,71	7,81	8,46	7,9 (SMA)
	SD	1,10	0,18	0,77	1,44	0,31	
	CV %	14,0	2,3	10,0	18,4	3,7	
2)	No.	47	6	13	22	3	
	Mean	17,70	28,50	28,76	27,46	28,00	
	SD	3,12	0,61	2,80	3,76	0,50	
	CV %	11,3	2,1	9,7	13,7	1,8	
3)	No.	52	6	13	26	4	
	Mean	8,44	8,30	8,21	8,49	9,23	
	SD	0,77	0,16	0,56	0,95	0,61	
	CV %	9,1	1,9	6,8	11,2	6,6	
4)	No.	51	6	12	27	3	
	Mean	12,28	12,82	10,34	12,53	12,06	12,8 (SMA)
	SD	1,34	0,29	0,25	1,15	2,21	
	CV %	10,9	2,3	2,4	9,2	18,3	
5)	No.	46	6	11	23	4	
	Mean	10,12	10,05	10,62	10,15	10,55	
	SD	0,94	0,43	0,70	1,11	0,70	
	CV %	9,3	4,3	6,6	10,9	6,6	
6)	No.	47	6	12	24	2	
	Mean	26,84	27,85	27,48	26,22	27,80	
	SD	2,06	0,73	1,55	2,28	-	
	CV %	7,7	2,6	5,6	8,7	-	
7)	No.	52	6	12	27	4	
	Mean	18,45	18,95	18,96	18,09	18,42	
	SD	1,74	0,59	1,76	2,02	0,91	
	CV %	9,4	3,1	9,3	11,2	4,9	
Mean No.		49	6	12	25	3	
Mean value		15,95	16,34	16,01	15,82	16,36	
Mean CV %		10,2	2,7	7,2	11,9	7,0 (6 results)	

TABLE XXII continued

(f) CREATININE (µmol/l)

		Overall	6/60	Jaffé with deproteinization	Rate methods	Stated value
1)	No.	39	6	26	6	
	Mean	112,99	107,67	116,26	102,60	106 (SMA)
	SD	17,25	5,43	18,55	16,90	
	CV %	15,27	5,04	15,96	16,47	
2)	No.	39	6	27	5	
	Mean	358,18	377,83	348,36	374,92	
	SD	41,66	6,05	46,34	25,24	
	CV %	11,63	1,60	13,30	6,73	
3)	No.	42	6	29	6	
	Mean	124,11	120,16	125,46	117,15	
	SD	16,80	4,62	18,32	13,81	
	CV %	13,54	3,84	14,60	11,79	
4)	No.	43	6	31	6	
	Mean	354,56	380,17	347,90	340,48	380 (SMA)
	SD	37,12	14,08	38,26	35,52	
	CV %	10,47	3,70	11,00	9,55	
5)	No.	40	6	28	5	
	Mean	156,73	156,83	155,33	160,85	
	SD	23,00	3,71	28,80	44,71	
	CV %	14,67	2,37	13,39	27,80	
6)	No.	41	6	28	6	
	Mean	333,87	348,33	326,24	343,97	
	SD	34,62	8,41	36,35	28,42	
	CV %	10,37	2,41	11,14	8,26	
7)	No.	45	6	32	6	
	Mean	612,07	702,33	595,02	641,92	
	SD	97,54	31,26	97,00	68,31	
	CV %	15,94	4,45	16,30	10,64	
Mean No.		41	6	29	6	
Mean value		293,22	313,33	287,80	297,41	
Mean CV %		13,13	3,34	13,67	13,03	

TABLE XXII continued

(g) GLUCOSE (mmol/l)

		Overall	Beckman	GO/PAP	Glucostrate	Stated value
1)	No.	50	17	14	10	
	Mean	5,19	5,07	5,31	5,28	5,8 (Beckman)
	SD	0,62	0,29	0,82	0,81	
	CV %	11,9	5,7	15,4	15,3	
2)	No.	47	16	15	7	
	Mean	9,02	8,66	8,99	9,69	
	SD	0,79	0,55	0,85	1,00	
	CV %	8,8	6,4	9,5	10,3	
3)	No.	52	17	16	11	
	Mean	5,34	4,88	5,56	5,53	
	SD	0,64	0,38	0,60	1,20	
	CV %	12,0	7,8	10,8	21,7	
4)	No.	49	16	15	9	
	Mean	10,03	9,73	10,34	10,44	10,2 (Beckman)
	SD	0,89	0,75	0,92	0,85	
	CV %	8,9	7,7	8,9	8,1	
5)	No.	51	15	18	11	
	Mean	5,22	4,90	5,24	5,75	
	SD	0,50	0,38	0,45	0,44	
	CV %	9,6	7,8	8,6	7,7	
6)	No.	48	16	14	9	
	Mean	10,77	10,47	10,70	11,16	
	SD	0,72	0,58	0,64	0,80	
	CV %	6,7	5,5	6,0	7,2	
7)	No.	52	16	17	10	
	Mean	3,24	3,16	3,30	3,46	
	SD	0,55	0,38	0,52	0,75	
	CV %	17,0	12,0	15,8	21,7	
Mean No.		50	16	16	10	
Mean value		6,97	6,70	7,06	7,33	
Mean CV %		10,7	7,6	10,7	13,1	

TABLE XXII continued

(h) ALBUMIN (g/l)

		Overall	ABA-100	Manual BCG	Stated value
1)	No.	46	4	38	
	Mean	36,06	36,63	36,17	43 (ABA-100)
	SD	3,48	5,44	3,24	38 (SMA)
	CV %	9,7	14,9	9,0	
2)	No.	47	4	39	
	Mean	39,09	41,50	38,53	
	SD	3,65	1,43	3,80	
	CV %	9,3	3,4	9,9	
3)	No.	51	4	43	
	Mean	35,88	38,90	35,30	
	SD	3,30	2,66	2,92	
	CV %	9,2	6,8	8,3	
4)	No.	48	4	42	
	Mean	36,19	37,36	36,18	44 (ABA-100)
	SD	3,22	3,94	2,63	38 (SMA)
	CV %	8,9	10,5	7,3	
5)	No.	46	4	38	
	Mean	31,02	33,74	30,76	
	SD	2,69	2,89	2,70	
	CV %	8,7	8,6	8,8	
6)	No.	46	4	38	
	Mean	31,69	33,78	31,41	
	SD	3,01	2,34	3,11	
	CV %	9,5	6,9	9,9	
7)	No.	49	4	41	
	Mean	27,72	31,53	27,31	
	SD	2,73	2,60	2,47	
	CV %	9,8	8,2	9,0	
Mean No.		48	4	40	
Mean value		33,95	36,24	33,67	
Mean CV %		9,3	8,5	8,9	

TABLE XXII continued

(j) CALCIUM (mmol/l)

		Overall	ABA-100	Manual CPC	Stated value
1)	No.	44	3	40	
	Mean	2,46	2,46	2,46	2,42 (ABA-100)
	SD	0,19	0,10	0,20	
	CV %	7,7	4,1	8,1	
2)	No.	42	3	38	
	Mean	2,72	2,66	2,72	
	SD	0,15	0,032	0,15	
	CV %	5,5	1,2	5,6	
3)	No.	46	3	42	
	Mean	2,48	2,39	2,48	
	SD	0,14	0,17	0,14	
	CV %	5,6	7,1	5,6	
4)	No.	40	3	36	
	Mean	2,77	2,85	2,77	2,9 (ABA-100)
	SD	0,16	0,055	0,17	
	CV %	5,8	1,9	6,1	
5)	No.	39	3	35	
	Mean	2,57	2,64	2,57	
	SD	0,12	0,046	0,12	
	CV %	4,7	1,7	4,7	
6)	No.	39	3	35	
	Mean	2,81	2,97	2,80	
	SD	0,17	0,13	0,16	
	CV %	6,0	4,4	5,7	
7)	No.	42	3	38	
	Mean	2,27	2,29	2,27	
	SD	0,19	0,046	0,20	
	CV %	8,4	2,0	8,8	
Mean No.		42	3	38	
Mean value		2,58	2,61	2,58	
Mean CV %		6,2	3,2	6,4	

TABLE XXII continued

(k) CHOLESTEROL (mmol/l)

		Overall	ABA-100	Manual enzymatic	LB reaction	Stated value
1)	No.	43	6	32	4	
	Mean	4,16	3,96	4,14	5,11	4,1 (ABA-100)
	SD	0,55	0,18	0,56	0,74	
	CV %	13,2	4,5	13,5	14,5	
2)	No.	42	5	32	4	
	Mean	5,88	5,78	5,79	6,67	
	SD	0,92	0,25	0,85	1,83	
	CV %	15,6	4,3	14,7	27,4	
3)	No.	46	6	35	4	
	Mean	4,01	3,84	4,00	4,47	
	SD	0,38	0,30	0,38	0,29	
	CV %	9,5	7,8	9,5	6,5	
4)	No.	45	6	34	4	
	Mean	5,12	4,97	5,16	5,58	4,9 (ABA-100)
	SD	0,49	0,41	0,56	0,58	
	CV %	9,6	8,2	10,9	10,4	
5)	No.	42	5	33	3	
	Mean	4,12	4,19	4,11	4,08	
	SD	0,39	0,29	0,43	0,13	
	CV %	9,5	6,9	10,5	3,2	
6)	No.	41	6	30	4	
	Mean	1,88	1,66	1,87	2,16	
	SD	0,29	0,19	0,26	0,39	
	CV %	15,4	11,4	13,9	18,1	
7)	No.	46	6	37	2	
	Mean	2,63	2,39	2,67	2,75	
	SD	0,28	0,36	0,26	-	
	CV %	10,6	15,1	9,7	-	
Mean No.		44	6	33	4	
Mean value		3,97	3,83	3,96	4,40	
Mean CV %		11,9	8,3	11,8	13,4 (6 results)	

TABLE XXII continued

(1) ALKALINE PHOSPHATASE

		ABA-100	Phenyl-phosphate	Phosphastrate	Stated value
1)	No.	5	6	32	
	Mean	108,60	11,48	44,81	129 (ABA-100)
	SD	10,83	2,13	6,74	
	CV %	10,0	18,6	15,0	
2)	No.	4	6	26	
	Mean	276,75	30,91	92,42	
	SD	19,19	2,27	13,26	
	CV %	6,9	7,3	14,3	
3)	No.	4	5	32	
	Mean	83,75	7,82	34,09	
	SD	11,67	1,54	5,44	
	CV %	13,9	19,7	16,0	
4)	No.	4	5	32	
	Mean	277,5	28,94	94,20	255 (ABA-100)
	SD	8,70	4,03	18,31	
	CV %	3,1	13,9	19,4	
5)	No.	2	3	29	
	Mean	75,70	8,71	30,64	
	SD	-	3,32	3,94	
	CV %	-	38,1	12,9	
6)	No.	3	3	26	
	Mean	152,67	12,75	49,85	
	SD	10,60	2,48	9,00	
	CV %	6,9	19,5	18,1	
7)	No.	3	2	30	
	Mean	55,67	11,25	45,30	
	SD	2,89	-	12,56	
	CV %	5,2	-	27,7	
Mean No.		4	4	30	
Mean value		147,23	15,98	55,90	
Mean CV %		7,7 (6 results)	19,5 (6 results)	17,6	

TABLE XXII continued

(m) ASPARTATE TRANSAMINASE

		ABA-100 (37°C)	Worthington Gilford (30°C)	Stated value
1)	No.	5	12	
	Mean	35,60	27,50	40 (ABA-100) (37°C)
	SD	4,34	5,76	
	CV %	12,2	20,9	
2)	No.	5	12	
	Mean	151,00	98,17	
	SD	6,24	9,99	
	CV %	4,1	10,2	
3)	No.	4	11	
	Mean	41,00	34,00	
	SD	4,32	5,42	
	CV %	10,5	15,9	
4)	No.	4	12	
	Mean	155,00	102,58	180 (ABA-100) (37°C)
	SD	4,83	16,16	
	CV %	3,1	15,8	
5)	No.	3	11	
	Mean	53,00	38,91	
	SD	4,41	7,78	
	CV %	8,3	20,0	
6)	No.	4	11	
	Mean	140,50	82,18	
	SD	16,52	15,24	
	CV %	11,8	18,5	
7)	No.	4	11	
	Mean	53,25	36,55	
	SD	5,38	6,23	
	CV %	10,1	17,0	
Mean No.		4	11	
Mean value		89,91	59,98	
Mean CV %		8,6	16,9	

TABLE XXIII. Analytical methods in use and incidence of "Wild" results in the SAIMR Quality Control Programme

Test/Method		Number of users	Mean number of results accepted	"Wild" results excluded (overall) High	Low	Total	Percentage of "Wild" result
SODIUM	6/60	6	6			Nil	Nil
	IL	22	21		1	1	0,7%
	EEL	27	21	1	3	4	2,7%
	Beckman Klina Corning-EEL	3	3			Nil	Nil
POTASSIUM	6/60	6	6			Nil	Nil
	IL	22	21			Nil	Nil
	EEL	27	20	7	4	11	7,3%
	Beckman Klina Corning-EEL	3	3			Nil	Nil
CHLORIDE	6/60	6	6			Nil	Nil
	Radiometer	25	24			Nil	Nil
	Schales	26	19	3	3	6	4,3%
BICARBONATE	6/60	5	5			Nil	Nil
	Harleco	46	36	3		3	1,2%
	AA-1	1	1			Nil	Nil
UREA	6/60	6	6			Nil	Nil
	Beckman	13	12			Nil	Nil
	Urease/Berthelot	32	25	2	5	7	3,9%
	Manual DAM	4	3		2	2	7,7%
CREATININE	6/60	6	6			Nil	Nil
	Jaffe' (End point)	37	29	3	7	10	4,7%
	Jaffe' (Rate)	6	6			Nil	Nil
	AA-1	1	1			Nil	Nil
GLUCOSE	Beckman	17	16			Nil	Nil
	Manual GO/PAP	19	16		1	1	0,9%
	Glucostrate	13	10	2	2	4	5,6%
	Copper reduction GO/PERID Centrifugal analysers ABA-100 6/60, 12/60	9	8			Nil	Nil
ALBUMIN	ABA-100	4	4			Nil	Nil
	Manual BCG	47	40	3	5	8	2,8%
	Salt fractionation 12/60 Centrifichem	4	4			Nil	Nil
CALCIUM	ABA-100	3	3			Nil	Nil
	Manual CPC	49	38	4	4	8	2,9%
	12/60	1	1			Nil	Nil
CHOLESTEROL	ABA-100	6	6			Nil	Nil
	Manual CHOD/PAP	40	33	3		3	1,5%
	Liebermann-Burchard	4	4	1		1	
	12/60	1	1			Nil	Nil
			Mean number of results submitted				
ALKALINE PHOSPHATASE	Phosphastrate	35	30				
	ABA-100 (PNPP - 37°)	5	4				
	PNPP-Rate (Manual)	4					
	Phenylphosphate	8	4				
ASPARTATE TRANSAMINASE	Worthington/Gilford (30°C)	12	11				
	ABA-100	5	4				
	Reitman-Frankel	10					
	"Transae"	5					
	Coulter "C-Zyme"	4					
	Boehringer/Gilford	3					
	Centrifichem	1					

number of participants (58) are attributable to two circumstances:-

a) Non-performance of the assay: for example, four of the five laboratories in the Eastern Cape Region do not perform bicarbonate assays aa part of the routine "electrolyte" profile.

b) A few individual methods not included in the breakdown: for example, chloride by the Mark I Autoanalyser; urea by a manual urease-glutamate dehydrogenase procedure.

Table XXIV compares the SAIMR figures with those obtained in the UK Scheme (1969 - 71)[105] and in the Wellcome Programme during the six-month cycle October 1977 - March 1978). With regard to the UK figures, the CV values for different methods (e.g. 2,69% for sodium by the EEL Flame Photometer) relate in each case to a single issue of control serum, whereas the overall figure (e.g. a CV of 2,14% for sodium assays) are average figures for the whole period. The "Autoanalyser" results for the UK include all Technicon systems and are not confined to the SMA 6/60.

Results for the Wellcome Programme were obtained during the participation in the Programme described earlier (Chapter 4); consequently only overall figures and figures for the single method actually used were obtainable.

7.5 Discussion

Whitehead, Browning and Gregory[105] noted that approximately 90% of participating laboratories in the UK Scheme returned the results for each distribution of serum. The same response rate was found

TABLE XXIV. Comparative performances of methods in the SAIMR and other external Quality Control Programmes (percentage coefficients of variation)

Test		SAIMR 1980		UK 1969 - 71*		Wellcome Programme 1977 - 78
SODIUM	Overall		2,17		2,14	1,56
	Auto-Analyser	(6/60)	1,08	(AA)	1,29	-
	EEL		2,83		2,69	-
	IL		1,36		-	1,67
POTASSIUM	Overall		4,3		3,98	2,60
	Auto-Analyser	(6/60)	2,5	(AA)	2,73	-
	EEL		5,0		4,05	-
	IL		3,2		-	2,77
CHLORIDE	Overall		2,68		2,56	2,68
	Auto-Analyser	(6/60)	2,40	(AA)	2,20	-
	Coulometric		2,13	(EEL)	2,86	2,40
	Schales		3,27		3,47	-
UREA	Overall		10,2		8,39	6,20
	Auto-Analyser	(6/60)	2,7	(AA)	5,67	-
	Manual urease	(Berthelot)	11,9		10,58	-
	Manual DAM		7,0		-	9,19
CREATININE	Overall		13,13		15,51	7,46
	Auto-Analyser	(6/60)	3,34	(AA)	6,75	-
	Manual		13,67		11,81	9,52
GLUCOSE	Overall		10,7		9,24	7,81
	Manual glucose oxidase		10,7		9,01	7,98
ALBUMIN	Overall		9,3		9,32	10,37
	Manual BCG		8,9		7,49	8,16
CALCIUM	Overall		6,2		5,16	5,99
	Manual CPC		6,4		-	4,94
CHOLESTEROL	Overall		11,9		10,11	13,94
	Manual L/B reaction		13,4		8,54	-
	Manual enzymatic		11,8		-	10,90
ALKALINE PHOSPHATASE						
	Kind and King		19,5		17,40	Assay not performed
	King-Armstrong				23,62	
	Phosphastrate		17,6		19,5	

*U.K. results for individual methods are for a single issue of control serum.

in the SAIMR Programme, 364 out of a possible 406 results being received (89,7%). The failure rate was noticeably higher for the smaller, more remote laboratories, as shown by the lower percentage of returns for methods and equipment coupled with such laboratories (e.g. EEL Flame Photometer, Schales chloride procedure). Only three of the 42 returns missing at the deadline for data processing subsequently arrived late.

In their discussion of the UK findings, Whitehead *et al.*[105] observed that the overall spread of results appeared to be due to a failure in precision rather than differences in the mean results of the various methods used. The same can generally be said of the SAIMR results, bearing in mind that the smaller numbers of results for many of the individual methods can increase the standard error of the mean. Among differences to be observed are the tendency towards lower sodium values by the EEL relative to the IL and the 6/60, higher bicarbonate results by the Harleco relative to the 6/60, lower results for the manual end-point Jaffé reaction for creatinine relative to the automated procedure (at high levels) and, as anticipated, higher values for cholesterol by the Liebermann-Burchard procedure relative to enzymatic methods. The tendency towards lower values for the manual BCG method for albumin, relative to the automated version, is an odd, unexplained finding; the mean difference of around 2,6 g/l is nearly twice the figure of 1,4 g/l found for the corresponding difference in one control specimen in the UK Scheme.

Considerable fluctuations in the overall CVs occur from one control specimen to another. This was also a feature of the UK

Scheme,[105] as illustrated below in the comparative findings for some of the observed ranges of CV values:

	SAIMR	UK
Potassium	2,0 - 8,4%	3,3 - 5,12%
Urea	7,7 - 14,0%	6,31 - 17,14%
Creatinine	10,37 - 15,94%	9,53 - 20,89%
Glucose	6,7 - 17,0%	7,68 - 13,52%

7.6 Performance of the methods investigated in the present work in SAIMR Branch Laboratories

The Quality Control Programme offered the opportunity for a "field trial" of the proposed standard methods developed in this work, and the concluding section of this chapter provides comments on the performances obtained.

It is evident from the figures for precision, and the incidence of wild results, that the IL Flame Photometer and the Radiometer Chloride Meter exhibit a much greater reliability than the older EEL Flame Photometer and the Schales titration method for chloride.

With regard to urea, the direct diacetyl monoxime method had an overall mean very close to that for the 6/60 and had an overall precision superior to that of the urease-Berthelot method. However, the introduction of the method appears to have encountered a strong psychological resistance in the Branch Laboratories, traceable to the use of a boiling water bath which is regarded as "archaic" by the younger generation of technologists (who have entered the profession since the era of copper-reduction glucose methods!).

The manual creatinine method appears to show non-linearity at

higher levels when read on simple colorimeters such as the Leitz; for this or other reasons the precision is inferior to both the UK and Wellcome figures.

Glucose estimations by the manual GO/PAP method show an improved precision over the Glucostrate procedure, which had become established in many Branch Laboratories upon the demise of the Folin-Wu method in the late 1960s. The manual CPC method for calcium is less precise in the Branch Laboratories than among participants in the Wellcome Programme, suggesting the need for greater care in the avoidance of contamination.

In the case of cholesterol and alkaline phosphatase estimations, the newer manual methods have overall precisions somewhat better than the older methods (Liebermann-Burchard, King-Armstrong) being replaced.

In overall summary, the performance of the standard methods in the Branch Laboratories, while not entirely as good as had been hoped, showed no calamitous shortcomings, so that these methods can be regarded as a satisfactory selection from those available at the present time. A number of laboratories were performing the colorimetric assays with "traditional" equipment (mouth operated pipettes, simple colorimeter), and improvement could be expected to follow the making-available of equipment for limited function automation.

CHAPTER 8

8. MEDICAL IMPLICATIONS OF ANALYTICAL PRECISION

The first detailed surveys on the interlaboratory performance of clinical chemical analyses were conducted in the decade following the Second World War (Belk and Sunderman,[106] 1947; Wootton and King,[107] 1953). While serious, indeed alarming inter-hospital differences in results on portions of the same material were reported, some time elapsed before their medical implication began systematically to be studied. Although Wootton and King[107] had early concluded that the differences were not explained by the use of different analytical methods, it was not until the widespread introduction of internal quality control procedures, originated by Levy and Jennings[108] in 1950, that the degree of variability of analytical results obtained by the same method within the same laboratory came to be appreciated.

An important undertaking in clinical chemistry during the past fifteen years has been the attempt to define what degree of analytical precision is desirable, in relation to the medical uses to which the laboratory findings are put.

Before considering some of the "criteria of acceptability" which have been put forward in answer to the foregoing question, it is necessary to consider the principal ways in which precision can influence the interpretation of analytical results on patients.

8.1 The confidence limit for the isolated result

If the factors giving rise to imprecision are assumed to be randomly distributed in a Gaussian pattern, the 95% confidence

limits for a numerical result will extend two analytical standard deviations above and below the value obtained. For a serum urea concentration reported as 5,0 mmol/l using a method with an analytical SD at that level of 0,5 mmol/l, there will be a one-in-twenty chance that the true value may lie outside the range of 4,0 - 6,0 mmol/l.

The probability that the result submitted is factually correct will, furthermore, depend on the ratio of the "reporting interval" (degree of "rounding off" of the result) to the SD. In the above example the reported figure of 5,0 implies that the true result lies between 4,950 and 5,049, effectively a tolerance of ± 0,05, or 0,1 SD. From a table of the integrated area under the standardised normal distribution curve for various deviations from the mean one can ascertain that, in the above case, the integral is equal to 0,0797; consequently there is a 92% probability that some other value (4,0, 4,1, 4,2 ..., etc.) would be more correct than the value of 5,0 actually submitted. This probability of incorrectness can be reduced either by widening the reporting interval or by reducing the analytical SD.

8.2 Interpretation of the result in relation to the range of reference values

When carrying out a survey to establish reference values for a particular analyte in a population, the variability found will be derived from two sources:

a) "Biological variability" (the parameter being sought).

b) "Analytical" variability.

As the two sources of variance are independent, their effect (again assuming normal distributions for each) will be additive. Consequently, the effect of analytical variation will be to extend the observed "normal range" beyond its true limits.[101]

For example, an analyte having a mean level of 100 arbitrary units per litre and a biological SD of 10 units would have a normal range (when defined as mean ± 2 SD) of 80 - 120 units per litre. However, if this range is established using a method with an analytical SD, at that level, of 5 units per litre the observed SD will be

$$\sqrt{10^2 + 5^2} = 11,18$$

The calculated normal range would then extend from 77,6 to 122,4 units per litre, a widening of approximately 12%.

The classification of a result as normal, high or low will therefore be subject to two sources of uncertainty, relating to the result itself (Section 8.1) and the reference range against it is to be assessed. An early study by Acland and Lipton[109] in 1967 demonstrated mathematically the reduced incidence of misclassifications ("diagnostic mistakes") which follows the improvement in analytical precision; however, their paper did not consider the influence of precision on the normal range itself and made no firm recommendations regarding acceptable limits of precision.

8.3 Interpretation of differences between serial results on the same patient

In the control of treatment, the clinician will frequently require to know if the latest result on a patient differs

significantly from an earlier finding reported on that patient. Campbell and Owen[98] have calculated that two results obtained on specimens collected under identical conditions have, on the basis of single analyses, to differ by 2,75 x the analytical SD for the change to be significant at the 5% level. For significance at the 1% level (i.e. 99 times out of 100) the limiting value for the difference is increased to 3,6 x SD.

Returning, by way of example, to the hypothetical urea value of 5,0 mmol/l given earlier, a subsequent value of 3,8 mmol/l would not show a significant change, since the ratio

$$\frac{5,0 - 3,8}{0,5}$$

equal to 2,4, is below the critical limit of 2,75.

8.4 Criteria of acceptability

8.4.1 Medical significance

The basis of this approach, pioneered by Barnett[110] in 1968, is to obtain a synthesis of opinions from clinical specialists as to what degree of analytical precision they deem necessary in the results submitted to them. Since the precision required is often related to the analyte level, it is usual for the data from such surveys to specify the required precision at various "decision levels" - for example, in the case of glucose, at levels of 2,2 mmol/l (detection of hypoglycaemia) and 6,7 mmol/l (2-hour level in the standard glucose tolerance test).

Barnett regarded the criteria of acceptability indicated by clinicians at various decision levels as corresponding to two

analytical standard deviations. Campbell and Owen[111] in 1969 and Barrett *et al.*[112] in 1979 based their assessments on the minimum differences between pairs of results which were regarded by clinicians as being significant, dividing these differences by 2,8 and 3,6 respectively to obtain the corresponding analytical standard deviation. In view of these differences in mathematical derivation, there are frequently wide differences in the criteria for "medically desirable precision" stemming from these surveys.

8.4.2 Analytical variability as a proportion of biological variability

An empirical formula put forward by Tonks[113] in 1963 proposed that the allowable limit of error should not exceed one quarter of the normal range, the full expression of the formula being

$$\text{allowable percentage error} = \frac{0{,}25 \times \text{normal range}}{\text{mean of normal range}} \times 100\%$$

Some confusion arose regarding what was implied by the allowable limit of error in Tonks' formula. If the interpretation of Gowenlock and Broughton[101] is followed, this error corresponds to $\pm$ 2 x analytical SD, so that the allowable analytical SD will be equal to one eighth of the normal range, or one half of the "measured" population SD.

A drawback to the soundness of Tonks' approach is that the measured population SD will depend on the magnitude of the analytical SD for the method used in its establishment, as shown earlier in Section 8.2. Consequently, later workers, following the same approach, have endeavoured to isolate the various

components of the measured population variance, so as to obtain the "true" biological SD.[114,115,116,117] Cotlove[116] in 1970 proposed that, to minimise artefactual increase in population variance, the analytical SD should be less than half that of the biological SD.

Differences between the Cotlove and Tonks criteria will depend on the analytical precision with which the "normal range" is originally established, as shown in the figures below for a hypothetical analyte present at a mean concentration of 100 units per litre.

Biological SD	Analytical SD	Measured SD	Acceptable CV% (Cotlove)	(Tonks)
10	5	11,18	5	5,59
10	10	14,14	5	7,07
10	15	18,03	5	9,01

The performance of a method used to establish a reference range can be tested for compliance with the Cotlove criteria by measuring the ratio

$$\frac{\text{analytical SD}}{\text{measured population SD}},$$

the limiting value for which is 0,447:

$$\left[\sqrt{1^2 - 0{,}447^2} = 0{,}894, \quad \frac{0{,}447}{0{,}894} = 0{,}5\right]$$

Further analysis of overall variance, using more elaborate experimental protocols and statistical techniques, can separate the variation occurring between persons (interindividual) from that occurring within persons (intraindividual).[115,118] It has been proposed[119,120] that for certain medical uses (such as serial

investigations on a single patient) the ratio of the analytical variability to the intraindividual variability is potentially more significant than the ratio to the composite biological variability, compounded from within-person and between-person sources.

8.4.3 Analytical precision in relation to achievable standards of performance

In place of criteria based on medical significance or on subtle physiological considerations, an alternative approach to the question of "acceptable" (as opposed to "desirable") limits of precision is to compare these features of a method with the performance of the assay by other methods and in other laboratories. A considerable volume of data relating to the "State of the Art" for clinical chemistry is available from a variety of sources, including the organisers of Quality Control Programmes, individual research workers and manufacturers of laboratory instruments and diagnostic kits.

8.5 Assessment of precision data obtained in the present work

Table XXV presents, for each of the twelve analytes covered, the following figures:-

1) Measured variability

a) The mean, SD and CV for the group of 40 blood donors (reference subjects). In the case of glucose and cholesterol, these values were recalculated after omission of the highest

TABLE XXV. Analytical precision in relation to various criteria of acceptability

(a) SODIUM

	Mean level (mmol/l)	SD (mmol/l)	CV%
(A) MEASURED VARIABILITY			
Reference subjects	140,90	2,061	1,46
Between-batch patients' specimens	139,55	0,784	0,56
Between-batch controls: M2	128,2	0,41	0,32
O2	138,3	0,52	0,37
Wellcome programme	136,33	1,2	0,9
(B) REQUIREMENT ACCORDING TO			
Barnett (1968)	130	2,0	1,5
	150	2,0	1,3
Campbell and Owen (1969)	136	0,9	0,7
Barrett (1979)	130	1,8	1,4
	145	1,8	1,3
Tonks' formula (1963)	140,9	1,03	0,73
Cotlove (1970)	139,4	0,5	0,4
Young (1971)	144,3	1,3	0,9
(C) STATE OF THE ART (50%)			
CAP survey (1977): Manual	130		1,2
Automated	130		1,2
Manual	150		1,1
Automated	150		1,1
Wellcome programme (1977)	136	1,5	1,1

TABLE XXV continued

(b) POTASSIUM

	Mean level (mmol/l)	SD (mmol/l)	CV%
(A) MEASURED VARIABILITY			
Reference subjects	4,348	0,309	7,11
Between-batch patients' specimens	4,055	0,069	1,59
Between-batch controls: M2	3,55	0,055	1,54
O2	4,02	0,041	1,02
Wellcome programme	4,20	0,049	1,17
(B) REQUIREMENT ACCORDING TO			
Barnett (1968)	3,0	0,25	8,3
	6,0	0,25	4,2
Campbell and Owen (1969)	4,5	0,083	1,8
Barrett (1979)	3,0	0,11	3,7
	5,0	0,11	2,2
Tonks' formula (1963)	4,348	0,155	3,6
Cotlove (1970)	4,12	0,14	3,4
Young (1971)	4,17	0,156	3,7
(C) STATE OF THE ART (50%)			
CAP survey (1977): Manual	3,0		2,5
Automated	3,0		2,6
Manual	6,0		1,7
Automated	6,0		1,7
Wellcome programme (1977)	4,2	0,08	1,9

TABLE XXV continued

(c) CHLORIDE

	Mean level (mmol/l)	SD (mmol/l)	CV%
(A) MEASURED VARIABILITY			
Reference subjects	102,78	2,154	2,096
Between batch patients' specimens	104,15	1,083	1,04
Between-batch controls: M2	88,7	0,52	0,58
O2	96,2	0,75	0,78
Wellcome programme	95	1,8	1,9
(B) REQUIREMENT ACCORDING TO			
Barnett (1968)	90	2,0	2,2
	110	2,0	1,8
Campbell and Owen (1967)	-	-	2,4
Barrett (1979)	85	1,1	1,3
	115	1,8	1,6
Tonks' formula (1963)	102,78	1,08	1,05
Cotlove (1970)	104,6	0,9	0,9
Young (1971)	108,9	1,2	1,1
(C) STATE OF THE ART (50%)			
CAP survey (1977): Manual	90	-	2,2
Automated	90	-	1,8
Manual	110	-	1,9
Automated	110	-	1,5
Wellcome programme (1977)	95	1,8	1,9

TABLE XXV continued

(d) BICARBONATE

	Mean level (mmol/l)	SD (mmol/l)	CV%
(A) MEASURED VARIABILITY			
Reference subjects	24,40	3,16	12,95
Between-batch patients' specimens	24,30	0,567	2,33
Between-batch controls: M2	19,3	2,10	10,9
O2	20,0	1,55	7,7
(B) REQUIREMENT ACCORDING TO			
Barnett (1968)	20	1,0	5,0
	30	1,0	3,3
Barrett (1979)	20	1,8	9,0
	35	1,1	3,1
Tonks' formula (1963)	24,40	1,58	6,5
Cotlove (1970)	27,3	0,8	2,9
Young (1971)	24,7	0,88	3,6
(C) STATE OF THE ART (50%)			
Wellcome programme (1977)	19	1,25	6,6

TABLE XXV continued

(e) UREA

	Mean level (mmol/l)	SD (mmol/l)	CV%
(A) MEASURED VARIABILITY			
Reference subjects	5,155	1,814	35,2
Between-batch patients' specimens	4,88	0,259	5,3
	9,96	0,261	2,6
Between-batch controls: M2	7,42	0,55	7,4
O2	17,74	0,16	0,9
Wellcome programme	13,16	0,48	3,65
(B) REQUIREMENTS ACCORDING TO			
Barnett (1968)	9,6	0,71	7,4
Campbell and Owen (1969)	5,8	0,21	3,6
	40	0,86	2,2
Barrett (1979)	9,0	0,89	9,9
Tonks' formula (1963)	5,155	0,91	17,6
Cotlove (1970)	4,8	0,54	11,1
Young (1971)	6,3	0,61	9,5
(C) STATE OF THE ART (50%)			
CAP survey (1977): Manual	9,6		6,6
Automated	9,6		3,7
Manual	17,86		5,8
Automated	17,86		2,8
Wellcome programme (1977)	13	0,50	3,8

TABLE XXV continued

(f) CREATININE

		Mean Level (µmol/l)	SD (µmol/l)	CV%
(A) MEASURED VARIABILITY				
Reference subjects		88,425	14,372	16,25
Between batch patients' specimens		106,42	4,09	3,84
		149,70	4,44	2,97
Between-batch controls:	M2	185,5	2,43	1,31
	O2	238,2	7,76	3,26
Wellcome progamme		246,46	7,3	2,96
(B) REQUIREMENTS ACCORDING TO				
Campbell and Owen (1969)		124	4,9	4,0
Barrett (1979)		50	7	14,0
		130	20	15,4
Tonks' formula (1963)		88,425	7,19	8,1
Young (1971)		92,8	3,9	4,2
(C) STATE OF THE ART (50%)				
CAP survey (1977):	Manual	133	-	8,1
	Automated	133	-	5,7
	Manual	442	-	5,4
	Automated	442	-	3,0
Wellcome programme (1977)		253	11	4,4

TABLE XXV continued

(g) GLUCOSE

	Mean Level (mmol/l)	SD (mmol/l)	CV%
(A) MEASURED VARIABILITY			
Reference subjects	4,418	0,619	14,0
Between-batch patients' specimens	4,83	0,225	4,66
	7,37	0,212	2,88
Between-batch controls: M2	4,63	0,18	3,9
O2	7,36	0,19	2,6
Wellcome programme	5,89	0,40	6,8
(B) REQUIREMENT ACCORDING TO			
Barnett (1968)	2,8	0,28	10
	5,6	0,28	5
	6,7	0,28	4,2
Campbell and Owen (1969)	5,6	0,13	2,3
	23,3	0,40	1,7
Barrett (1979)	2,2	0,07	3,2
	6,6	0,18	2,7
Tonks' formula (1963)	4,418	0,31	7,0
Cotlove (1970)	5,25	0,25	4,8
Young (1971)	5,24	0,18	3,5
(C) STATE OF THE ART (50%)			
CAP survey (1977): Manual	4,2		4,2
Automated	4,2		3,8
Manual	5,6		4,1
Automated	5,6		3,6
Manual	6,7		4,0
Automated	6,7		3,4
Wellcome programme (1977)	5,96	0,25	4,2

TABLE XXV continued

(h) ALBUMIN

		Mean level (g/l)	SD (g/l)	CV%
(A)	MEASURED VARIABILITY			
	Reference subjects	43,70	2,928	6,70
	Between-batch patients' specimens	38,10	0,678	1,78
	Between-batch control: M2	35,08	3,10	8,9
	O2	36,82	2,34	6,4
	Wellcome programme	38,63	0,44	1,14
(B)	REQUIREMENT ACCORDING TO			
	Barnett (1968)	35,0	2,5	7,1
		30,0	1,1	3,7
	Barrett (1979)	52,0	1,8	3,5
	Tonks' formula (1963)	43,70	1,46	3,35
	Cotlove (1970)	42,0	1,5	3,6
	Young (1971)	48,7	0,9	1,8
(C)	STATE OF THE ART (50%)			
	CAP survey (1977): Manual	35,0		4,9
	Automated	35,0		3,4
	Wellcome programme (1977)	36,5	1,7	4,7

TABLE XXV continued

(j) CALCIUM

	Mean level (mmol/l)	SD (mmol/l)	CV%
(A) MEASURED VARIABILITY			
Reference subjects	2,364	0,0736	3,11
Between-batch patients' specimens	2,160	0,0449	2,08
Between-batch controls: M2	2,53	0,062	2,4
O2	2,99	0,067	2,2
Wellcome programme	2,481	0,059	2,4
(B) REQUIREMENT ACCORDING TO			
Barnett (1968)	2,75	0,062	2,3
Campbell and Owen (1969)	2,70	0,021	0,8
Barrett (1979)	2,10	0,018	0,9
	2,60	0,021	0,8
Tonks' formula (1963)	2,364	0,037	1,6
Cotlove (1970)	2,55	0,04	1,6
Young (1971)	2,47	0,046	1,9
(C) STATE OF THE ART (50%)			
CAP survey (1977): Manual	2,0		3,2
Automated	2,0		2,4
Manual	2,75		3,2
Automated	2,75		2,2
Wellcome programme (1977)	2,48	0,07	2,8

TABLE XXV continued

(k) CHOLESTEROL

	Mean level (mmol/l)	SD (mmol/l)	CV%
(A) MEASURED VARIABILITY			
Reference subjects	5,162	0,878	17,0
Between-batch patients' specimens	4,843	0,118	2,44
Between-batch controls: M2	3,46	0,096	2,8
O2	2,64	0,082	3,1
Wellcome programme	2,933	0,046	1,6
(B) REQUIREMENT ACCORDING TO			
Barnett (1968)	6,48	0,52	8,0
Campbell and Owen (1969)	6,73	0,12	1,8
Barrett (1979)	6,5	0,18	2,8
Tonks' formula (1963)	5,162	0,44	8,5
Cotlove (1970)	5,31	0,44	8,3
Young (1971)	5,29	0,39	7,3
(C) STATE OF THE ART (50%)			
CAP survey (1977): Manual	6,48		5,0
Automated	6,48		4,3
Wellcome programme (1977)	3,27	0,22	6,7

TABLE XXV continued

(1) ALKALINE PHOSPHATASE

		Mean level (U/l)	SD (U/l)	CV%
(A)	MEASURED VARIABILITY			
	Reference subjects	22,928	6,11	26,65
	Between-batch patients' specimens	24,25	0,90	3,7
		48,10	1,14	2,4
	Between-batch controls: M2	51,95	2,54	4,9
	O2	38,55	2,06	5,3
(B)	REQUIREMENT ACCORDING TO			
	Barrett (1979)	(Normal)		4,3
		(2 x normal)		3,6
	Tonks' formula (1963)	22,93	3,06	13,3
	Young (1971)	(Normal)		11,6
(C)	STATE OF THE ART (OVERALL)			
	CAP survey (1978)			
	(Phenolphthalein phosphate, 37°C)			8,3
	(PNPP/DEA, 30°C)			8,1

TABLE XXV continued

(m) ASPARTATE TRANSAMINASE

		Mean level (U/l)	SD (U/l)	CV%
(A) MEASURED VARIABILITY				
Reference subjects		17,55	4,78	27,3
Between-batch patients' specimens		12,92	0,91	7,2
		29,80	1,05	3,5
Between-batch controls:	M2	42,23	3,41	8,1
	O2	37,08	1,57	4,2
(B) REQUIREMENT ACCORDING TO				
Campbell and Owen (1969)		(Normal)		4,2
		(8 x normal)		2,4
Tonks' formula (1963)		17,55	2,4	13,7
Cotlove (1970)		(Normal)		13,1
Young (1971)		(Normal)		9,9
(C) STATE OF THE ART (OVERALL)				
CAP survey (1978) (Spectrophotometric, 30°C)				10,3

and lowest result, to eliminate the influence of the single outlying high result in each case.

b) Figures relating to the three separate assessments of analytical variability, namely:

i) Between-batch duplicated analyses on patients' specimens (Table XVIII).

ii) Between batch analyses of the two Wellcome controls, M2 and O2 (Table XXI).

iii) Performance in the Wellcome Programme, for the ten analytes taken through the programme (Table VII).

Only figures relating to the manually performed assays are presented in the above data.

2) Required degree of analytical precision

a) Criteria proposed on medical grounds by Barnett,[110] Campbell and Owen[111] and Barrett *et al.*[112] In the case of chloride, the figure given by Campbell and Owen was contained in an earlier paper in 1967,[98] which only presented the mean desired CV without specifying the level.

b) The figures obtained by the application of Tonks' formula[113] to the reference range data given earlier in 1.a). The arbitrary upper limit of 10% for the analytical CV, proposed by Tonks, was not adhered to in those cases (urea and enzyme assays) where figures greater than 10% were derived.

c) The figures of Cotlove[116] and Young[117] in which the desirable limit of analytical precision was calculated as half the composite biological SD. In both cases the CV figures

have been calculated from the mean values of the experimental subjects given in the original published data.

3) "State of the Art" figures

a) The CAP survey figures relate to data derived from the Quality Assurance Service of the College of American Pathologists. For analytes other than the two enzymes, the survey data used covered the period from January 1976 until May 1977.[121] The figures relating to alkaline phosphatase and aspartate transaminase are taken from a survey of five enzymes conducted between May and December 1978.[122]

b) The performance of various assays in the Wellcome Control Programme are taken from data published by the Association of Clinical Biochemists in 1978,[123] and relate to the six-month period July - December 1977. The above report did not provide the actual mean levels for the analytes during this period, but only the extremes of the range; the coefficients of variation given in Table XXV have therefore been calculated from the end-of-cycle figures (March 1978) for these mean values.

The State of the Art figures from both these sources are presented for different levels of performance (i.e. top 10%, top 50%, overall average). The figures given in Table XXV are the 50% cut-off figures, and thus represent the minimum level of performance for the upper half of the participating laboratories.

8.6 Discussion

The most striking feature of the figures given in Table XXV is

the wide spread of values for the analytical precision found by the various experimental protocols adopted (Sections A), and for that required according to various criteria (Sections B).

There is generally a closer agreement between the State of the Art figures, taking into account the fact that the Wellcome Programme results include both manual and automated assays, and also the existence of differences in the mean analyte levels covered by the CAP and Wellcome Programmes.

The CAP survey results at differing analyte concentrations demonstrate the reduction in CV with higher analyte levels, noted by Broughton and Annan;[124] the only exception being for manual calcium assays where there was no reduction in CV (3,2%) on moving from a level of 2,0 mmol/l to one of 2,75 mmol/l. The precision for different analyte levels for the two control sera M2 and O2 are not so consistently related, higher analyte levels being associated with higher CV values for sodium, chloride, creatinine and aspartate transaminase.

With regard to the differences between the various criteria of acceptability for analytical precision, it has previously been noted that these can originate from the different types of questionnaire submitted to clinicians, and varying mathematical treatments of the data received. Notable examples of such differences are seen in the precision requirements for urea assays given by Campbell and Owen (CV of 3,6% at a level of 5,8 mmol/l), and Barrett *et al.* (9,9% at a level of 9,0 mmol/l). In other cases, the different criteria of acceptability reflect large differences in the analyte level at which the assessments are made.

The surveys of Cotlove[114] in 1970 and Young[117] in 1971 involved, in the former case, a group of 68 subjects, with analyses carried out intermittently over a three-month period, and in the latter case a group of only nine subjects, with all analyses carried out on a single day at the end of the survey period. Differences in the values for acceptable precision, based on assessment of the composite biological variation, are substantial in the case of sodium and albumin, and reflect differences both in the overall measured variability and in the analytical component of variability found in the two surveys.

The estimations of analytical variability, given in Section A for each analyte, represent three different experimental approaches The duplicate assays on patients' specimens provide data on the short-term reproducibility over two consecutive days for twenty different sera with differing analyte levels; the assays on control sera M2 and O2 provide data on the medium-term reproducibility over 2 - 3 weeks for six replicate assays at two analyte levels, while the Wellcome Programme results provide data on the long-term reproducibility, over a period of up to six months, for 2 - 5 replicate assays at four different levels.

Under differing circumstances of patient investigation, each of the three approaches could be regarded as appropriate for the determination of analytical precision, but it is a feature of all the twelve analytes studied that the different protocols give different indications of precision and that there is no consistent relationship between them. Contrary to expectation, the long-term precision was in a number of cases superior to both the short- and medium-term precision, this being especially noticeable in the case of albumin and cholesterol.

One is led to the conclusion that, while the average results from large numbers of assays from many laboratories can consistently support certain generalisations (for example, that higher analyte levels are associated with lower analytical CV values, or that long-term precision is inferior to short-term precision), there is a degree of unpredictability about the performance of small numbers of assays in a single laboratory.

Broughton and Annan[124] encountered such a phenomenon in the estimation of urea by the AutoAnalyser, finding that the between-batch precision for twenty daily assays per month on a serum pool, at a level of 160 mg/100 ml, varied from month to month between about 1,0 and 3,5 mg/100 ml.

Of the twelve analytes under consideration in this work, there were two (potassium and creatinine) for which the worst analytical precision displayed was able to meet the most stringent criteria of desirable performance. If the between-batch reproducibility on patients' specimens is measured against the criteria of Young[117] (where the biological variability was assessed following a similar protocol for the determination of analytical variability), a satisfactory performance is found for sodium, potassium, chloride, bicarbonate, urea, creatinine albumin, cholesterol, alkaline phosphatase and aspartate transaminase. In the case of chloride, creatinine (at normal levels) and albumin the precision was, however, only narrowly within the required limits. The estimation of glucose (at normal levels) and of calcium failed to meet Young's criteria.

For all assays taken through the Wellcome Programme, apart from glucose, analytical precision met or bettered the State of the Art figures relating to the Wellcome Programme itself, or the manually performed assays in the CAP survey. As was noted earlier, in Chapter 4, the poor performance of glucose in the Programme was due to a single wild result in the series.

In concluding this chapter, mention needs to be made of two criticisms which have been levelled against the entire concept of attempting to establish criteria for desirable or acceptable performance of clinical chemical tests.

Since different clinical purposes can require different levels of performance, the IFCC Expert Panel on Quality Control[125] have expressed the view that "it is unrealistic to stipulate a single set of values of the performance characteristics which would make a method acceptable for all purposes in clinical chemistry".

Whitehead[126] has commented that "there are dangers in documenting acceptable variances for routine clinical chemical determinations, because such standards, once they have been decided on, result in a laboratory failing to progress further in trying to improve their variance".

CHAPTER 9

9. CONCLUDING SUMMARY

The introduction of instrumental methods of analysis into clinical chemistry was remarkable in combining two benefits - increased reliability and increased speed - which, for many fields of human endeavour, are often mutually exclusive. Criticisms made of some of the subsequent developments in the application of these methods, in particular their incorporation into large and costly machines for the performance of unsolicited multiple analyses, should not diminish appreciation of their fundamental value in the present-day laboratory.

The decade of the 1950's, culminating in the introduction of the Technicon AutoAnalyser, can be looked back on as the most eventful and exciting in the short history of clinical chemistry. It was during that period that the techniques of chromatography and electrophoresis, the utilisation of radioisotopes, and major advances in laboratory methods for enzyme, hormone and acid-base studies all combined to transform clinical chemistry into the powerful diagnostic weapon it is today. In the midst of this stimulating activity, the development of "machines" to carry out such routine and mundane assays as blood urea and glucose could only be welcomed!

Whitehead[127] has described the following four phases of development which are frequently associated with the application of technological innovations in medicine. In the particular context of the application of computers in clinical laboratories, Whitehead considered (in 1978) that development was somewhere

between phases three and four, but in the application of modern analytical instrumentation the contemporary situation is rather further back - possibly between phases two and three. The four phases are:

1) "The over-optimistic introductory period led by enthusiasts."

Whitehead himself captured the mood of this initial phase when, in 1966, he endeavoured to predict the nature of clinical chemistry in the year "1984". He wrote:[128]

> "Medicine is moving from an era when data was expensive and therefore limited to an era when it is cheap and challenging. It may be that it is in the area of laboratory screening that developments in preventive medicine may take place."

2) "A phase of developing pessimism as the limitations and full costs of the innovation are realised."

As expressed in the moderate words of Büttner:[129]

> "Fascinated by new developments in technology, clinical chemists have in recent years paid too much attention to improved production in terms of quantity and quality. The time has come when special attention must be given to the information content of laboratory data and to improved communication with clinicians."

And more dramatically by Marks:[4]

> "What evidence there is suggests that while clinical biochemistry (and other laboratory tests) are invaluable or indispensible in a small but important group of subjects, they are in the majority of patients only moderately

or slightly helpful. Sometimes they may be frankly misleading and counter-productive."

3) "A recovery phase where a balancing of the claims of phases one and two is achieved."

4) "Finally, a fourth phase where the innovation is applied at its correct level."

It is the contention of the present author that the performance of biochemical tests on a discretionary basis, using limited function automation, could, for many laboratories, represent this fourth phase.

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APPENDICES

Medical Laboratory Technology (1971) 28, 191–196

Problems Associated with the Large-scale Centralization of Routine Biochemical Work †

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To permit the more effective use of high-output automatic analytical equipment and of computer-based data processing systems, proposals have been put forward for the performance of all the routine biochemical work of a group of hospitals in a central "factory" laboratory. Various advantages and disadvantages of such an arrangement are considered, particular consideration being given to human factors. Possible developments in laboratory organization which maintain the principle of decentralization are also discussed.

INTRODUCTION

In common with the other branches of clinical pathology, clinical chemistry has during the whole of its history been extending its activity in response to 2 main stimuli: (1) The increasing popularity of established tests; (2) the increasing range and complexity of tests requested.

Thus the hospital biochemist has had 2 major problems; undertaking an increasing workload of "routine" investigations, and introducing "new" tests.

While methodological advances have often provided simplified methods for the existing work (for example, the use of the flame photometer in place of chemical methods for sodium and potassium estimation), much of the developmental work at present is concerned with new estimations which are technically difficult, or time consuming, or dependent on sophisticated instrumentation.

The stimulus for the introduction of new chemical tests is spearheaded by the clinicians who supply work to the laboratory. By the nature and organization of medical education, consultants and registrars who initiate biochemical work have received their training in academic departments where it was possible to investigate and manage the rarer case by calling upon the more uncommon laboratory tests. Such persons will regard with frustrated irritation any limitation of tests of established value but more technical difficulty.

Historically, this second problem became acute at an earlier stage than that posed by increasing workloads. There developed, in consequence, in many parts of the world an unofficial system of reference of specialized work to a small number of undertaking centres. This process operated at first on what became known as the "old boy" system, but in some areas regional inter-laboratory co-operation ensured the setting up of arrangements on a regular, semi-official basis (Harkness & McGowan, 1966).

The centralized performance of specialized, non-urgent work by such means is considered to be entirely sensible and practical. An example local to the author is the per-

† Based on a paper presented at the Second National Congress of the Society of Medical Laboratory Technologists of South Africa, Johannesburg, August 1970.

formance at this Institute of serum protein-bound iodine estimations for a large area of Southern Africa including, at the present time, both Rhodesia and Zambia.

As long as routine biochemical analyses were performed by straightforward manual methods there was little prospective advantage in developing further the principle of centralized performance of biochemical work. However, in the last decade the advent of the AutoAnalyzer* and the more recent discrete automatic analysis systems has transformed the whole situation. The organization of the larger laboratory is tending more and more towards subdivision into a "factory" or "high-output" section, which carries out up to 20 routine analyses in batches by automatic methods, and a number of smaller sections which perform the remaining tests by manual or part-automated methods.

The creation of a central factory laboratory to process *all* the routine, automated biochemical work for a region comprising several hospitals has been proposed by a number of workers (Muir, 1966; Goldberg & Kind, 1968). Such an arrangement offers several advantages from the economic and organizational points of view. There are also many potential problems, which could constitute serious disadvantages. This paper is concerned particularly with a consideration of these latter factors.

The advantages of centralization

(1) *The more efficient use of costly equipment.* (a) If one imagines 2 hospital laboratories, A and B, each having an AutoAnalyzer set aside for non-urgent analyses (uric acid, cholesterol, total protein, etc.) and functioning only 8 hr/day, it is evident that a single machine in one of the centres could process the combined workload within the limits of an extended working day, employing technicians on a "shift" system. (b) Alternatively, if 6 hospital laboratories each possess an AutoAnalyzer which is used for the daily performance of 5 separate non-urgent tests which cannot be grouped together for multi-channel performance, then each analyser will suffer a substantial period of "down time" on switching from one estimation to another. Grouping of all the analysers in one centre, to which all the work is submitted, will allow each machine to operate continuously for its own particular estimation and would, in fact, free one machine entirely for use for some additional purpose. (c) Such a pooling of workloads would be still more desirable, if not essential, for the economic use of the very high output devices such as the Technicon S.M.A. 12/60* or the Vickers Multi-Channel 300†. Computer based data processing systems, particularly those operating "on line", are designed for conditions of almost continuous operation of such instruments.

(2) *Improvement in uniformity and quality of work.* (a) If, in the 2 laboratories previously considered, "A" is carrying out an estimation by a manual method and "B" by an automated method there may, and very often will be differences in precision, accuracy and range of normal value between them. These differences could be significant upon the patient being transferred from one hospital to the other. (b) Quality control methods (for example, the use of control sera or of cumulative sum techniques) can be used liberally and effectively with large automated batches of tests without subjecting the staff to any great extra pressure of work.

(3) *The provision of a more satisfactory environment and improved facilities for research, development and staff training.* It is envisaged that the peripheral laboratories in a group would maintain a local service for emergency tests, carried out in a small residual laboratory. Tests carried out would probably include blood glucose, electrolytes, amylase, neonatal bilirubin and so forth. This service would be operated by a qualified technician who would supply results directly to the clinician concerned.

The medical and scientific staff, research workers and technicians in training would all be concentrated in the central laboratory, as also would the bulk of the scientific equip-

* Technicon Instruments Corporation, Tarry Town, New York, N.Y., U.S.A.

† Vickers Ltd., Medical Group, Vickers House, Millbank, London, S.W.1.

ment. Items of equipment could then more readily be shared among research workers, and training schemes for all grades of staff could be operated regularly without the difficulties caused by geographical dispersal.

The problems of centralization

Some counter-arguments to centralization of routine biochemical work are as follows.

Technical and organizational problems. (a) The operation of automation in the laboratory is dependent on the continuous smooth working of highly complicated machines. The greater the work load of an AutoAnalyzer, the shorter the working life of such components as manifolds and dialyzer membranes. But with use extended to the full working day of continuous operation, less time would be available for routine maintenance and replacement.

Under these conditions, too, it would be almost impossible to "catch up" with the back-log of work on an analyser should one suffer a breakdown causing it to be out of action for a day or two. The economies made by centralization could thus be considerably offset by the need to duplicate much of the equipment, i.e. by keeping several AutoAnalyzer modules and made-up manifolds ready for immediate use as replacements.

(b) Equally vital is the need for a smooth-running system for specimen delivery, on the input side, and communication of results, on the output side. Both activities have so far defied automation, so that the large sophisticated laboratory, extensively automated in its internal operations, is still dependent on porters and van-rounds, and telephone and postal services. Future developments in population, town planning, traffic control, etc., could have serious effects on any transportation system based on today's circumstances.

In relation to the foregoing, the arguments are particularly relevant to any computer-based data processing system. Here one is utterly dependent on the output devices; it is possible for hundreds of the day's results to be "tied up" in the computer, completely inaccessible unless put out on the teleprinter. The problems inherent in long distance off-line linkage for access to a computer have been amply described (Whitby, Proffitt & McMaster, 1968).

(c) It is virtually impossible to lay down an entirely rigid programme for the batch performance of tests in daily, twice weekly or less frequent batches. Senior clinicians are aware of the fact that, prior to the advent of the AutoAnalyzer, simplified manual methods for the majority of routine estimations had been developed (King & Wootton, 1956) which could be carried out in 1 hr or so and thus could always provide them with a result on the same day. To deny them this service now would be widely regarded as a retrograde step, and could lead to the more affluent clinical units setting up their own service laboratories to meet their needs.

Depending on the idiosyncrasies of the local clinicians, therefore, it will be necessary to maintain a range of manually performed estimations for immediate use in special circumstances. However, it is generally accepted that, of all analyses, the infrequently performed manual method has the lowest standard of performance. Thus there could develop the extraordinary situation where emergency work performed on the acutely ill patient, for example, would be carried out with the worst degree of precision and accuracy.

Where, on the other hand, the laboratory retains responsibility for processing all its local workload it is often possible to be more flexible and put up a batch of tests including the urgent specimen outside the "set" operating times for the analysis concerned.

Problems of staffing and recruitment. Human factors have not hitherto been considered but in many ways they are the most important. Some regions of concern here are the following:

(a) Extension of the working day to 18 hr or more would require a late-shift of technicians working in the evening. This would interfere not only with their social lives but with their educational progress, since technicians rely much on evening class study for the attainment of their advanced qualifications.

(b) The everyday work of junior technicians would consist largely of tending the automatic analysers. The in-service training offered by the factory laboratory would fall short of the requirements of the qualifying examinations such as, for example, the National Diploma in Chemical Pathology administered by the Department of Higher Education in South Africa. This, and similar examinations elsewhere, require a sound knowledge of the theory and practice of many types of analytical method. Whereas in the past it was customary for the student to come from the small branch laboratory to the central laboratory for pre-examination experience, the reverse movement could be more profitable to him nowadays.

It is difficult to foresee the long-term solution to this problem, but in the short-term it is likely that the better type of school-leavers might be dissuaded by their career advisors from entering the profession of Medical Laboratory Technology at all.

The value of a laboratory. Finally, there is the whole question of the consultative function of the laboratory in relation to the clinical staff of the hospital or hospitals it serves, and the amount of control which the laboratory head and his senior staff should exert over the nature and number of investigations undertaken. Already there is an atmosphere of capitulation, with the philosophy of the mass performance of a wide battery of tests as an "admission profile" or indeed on every specimen received, gaining ground. The subject is highly controversial, but it can be predicted that once the laboratory abandons any form of control over requests and becomes subjected to unrestricted calls for investigations it is unlikely ever to re-establish control in the future.

The alternative to centralization

If the alternative policy to that of centralization is to be maintained, certain things can be done to improve the quality of the service given.

The introduction of work-simplified methodologies and equipment. Concurrently with the development of the AutoAnalyzer, the last 10 years have seen great advances in methods and devices for speeding-up and streamlining manual methods of analysis. The success of these techniques of "semi-automation" has provided the groundwork for the present upsurge of integrated discrete analytical systems. Some of the important developments in this field are the following:

(a) The use of automatic balances, dispensers, diluters, burettes, flow-through colorimeters, etc., have made it possible to achieve, even in the small laboratory, a state of "limited function automation" (Hardy, 1968) in which the various measuring operations are greatly speeded up and errors due to fatigue, boredom and lack of skill largely eliminated.

(b) The dispensing with deproteinization as a pre-requisite for many biochemical tests and consequent development of "single tube" methods have been eagerly exploited by the makers of test kits. Thus removal of protein plays no part in available methods for sodium, potassium, chloride, bicarbonate, urea, calcium, bilirubin, cholesterol, and enzyme determinations based on continuous monitoring of the reaction-rate.

(c) The availability of ready-made concentrated solutions for many standard analytical procedures can result in considerable time-saving by technical staff.

The introduction of uniform methods of work performance and quality control throughout all the peripheral laboratories of a group. Quality control systems can readily be introduced into the small laboratory (Padmore, 1969), often with a greater degree of practical interest for the operator.

DISCUSSION

The present growth rate in the work of hospital biochemistry laboratories involves a doubling of the workload approximately every 5 years, and predictions made on this

basis in the early 1960s (Whitby, 1963) have been fulfilled. One can confidently predict now that the annual throughput of estimations in several large laboratories will exceed the million mark during the coming decade.

Several surveys of the impact of automation on the modern clinical laboratory have appeared in recent years (for example, Nelson, 1969). The authors of these reviews appear in general to be stimulated rather than depressed by the present situation, and to express cautious optimism for the future. In particular, it is claimed by them that automation of analytical methods, and the computerized data processing of results can bring about improvements in productivity with reduction of costs, and a saving in technicians' time allowing their greater participation in development and research work.

Very recently, however, a faint note of disquiet has been voiced in certain quarters. During the early part of 1970 an interesting correspondence has taken place in the pages of the *Lancet*, initiated by a letter from Dr R. D. Eastham (1970) on "Inflation in the laboratory". A number of pathologists have expressed their concern over the contemporary laboratory scene.

A major cause of this concern is the growing lack of contact between the laboratory and the patient, with a growing lack of understanding and appreciation of each other's difficulties between laboratory and clinical staff.

In spite of the enormous increase in work output it is still often the case that a single person (frequently a quite junior graduate) is responsible for signing out the laboratory reports on any particular day. Through simple weight of numbers, a high proportion of reports receive only a cursory examination. A recent paper in this journal (McSwiney & Woodrow, 1969) has suggested that, even with very careful organization, approximately 3% of everything that one does in the laboratory is a mistake. The issuing of laboratory results without careful scrutiny, involving in each case a consideration of clinical history and previous laboratory findings, must be considered highly irresponsible.

It is worth considering if there is not an "optimum" size and workload for any single laboratory, corresponding to the economic concept of an optimum population for an area. Increase in the workload beyond such a level will make for an unwieldy and unmanageable unit, despite all the aids provided by modern technology, in the same way that increase in the supply of substrate provided to an enzyme beyond an optimal level can frequently inhibit the enzyme activity.

CONCLUSIONS

The continuous increase in the workload of hospital clinical chemistry departments has given rise to several problems in the past 20 years.

The initial problem, the increase in analytical work, has in large measure been solved by the introduction of mechanization. Subsequently, the problem of dealing with the greatly increased amount of paper work is in the process of being solved by the development of computer-based data processing systems.

However, the factory-like conditions of the present day laboratory, with deterioration or severance of relations between the laboratory and the hospital, now presents a set of problems to which no ready solution is apparent.

It is suggested that the growth of any single laboratory should not be allowed to proceed beyond an optimum level, which allows enjoyment of the benefits of modern technology but avoids the detrimental features of overloading. To this end, instead of centralization we should be moving towards the expansion of existing small laboratories and the creation of new laboratories.

Until such time as the growing workload of these laboratories justifies the introduction of full automation, use can be made of work-simplified systems or limited function automation. During this interval, careful thought will need to be given to the whole subject of training and qualification of technologists in the field of clinical chemistry.

(The statements and opinions set out in this paper are based extensively on the author's experience in the United Kingdom, and are independent of the official views and policies of the South African Institute for Medical Research.)

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MICRO DETERMINATION OF SERUM ALKALINE PHOSPHATASE USING PHENOLPHTHALEIN MONOPHOSPHATE AS SUBSTRATE

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SUMMARY:

A scaled-down version of the General Diagnostics 'Phosphastrate Alkaline' kit method is described and evaluated with regard to within-batch and between-batch precision. Results read off a phenolphthalein standard curve correlated well with those obtained by a fully automated procedure using p-nitrophenyl phosphate as substrate.

The phenolphthalein monophosphate method for alkaline phosphatase determination introduced by Babson, Greeley, Coleman and Phillips (1966) is generally performed by means of the reagent kit ('Phosphastrate alkaline') marketed by the General Diagnostics Division of Warner Lambert Company. The kit method follows the original published procedure with regard to specimen and reagent volumes, leading to a final volume for colorimetry of over six millilitres.

The present availability of low-volume flow-through absorptiometers and micro-volume semi-automated dispensers makes it possible to effect a five-fold reduction in these volumes, resulting in considerable reagent economy. Further economies can be effected through use of the following measures:-

1) Preparation of the working substrate by dilution of the 'stock' substrate available for use on automated analysers.
2) Preparation of the alkaline colour stabilizing reagent as formulated by Babson et al.
3) Reading of results from a phenolphthalein standard curve (as described in the original published method) instead of from a standard curve based on the activity of commercial control serum.

In the present study, readings were made on the Gilford Stasar III micro-sample colorimeter while specimen measurement was by means of a 20 microlitre SMI micropetter.

MATERIALS AND METHOD

1) **Working Substrate.** This is prepared immediately before use as 1/26 dilution of 'phosphastrate alkaline' substrate (General Diagnostics). A volume sufficient for eleven tests plus a reagent blank is prepared by diluting 0,1 ml of the stock substrate with 2,5 ml of water; greater or smaller volumes can be used depending on the batch size.
2) **Colour Stabilizer.** Dissolve 9,30 g Na_3PO_4. 12 H_2O and 13,48 g Na_2HPO_4. $2H_2O$ in water and make up to a litre with water. For use in the micro-assay of serum, the reagent can conveniently be stored in a 1,0 ml volume Oxford pipetter, at room temperature.
3) **Stock Solution of Phenolphthalein (2,5 mmol/litre).** Dissolve 79,6 mg phenolphthalein in 50 ml ethanol in a 100 ml volumetric flask and dilute to 100 ml with water.
4) **Phenolphthalein Standard Curve (Babson et al, 1966).** Dilute 1,0 ml of the above stock phenolphthalein solution to 50 ml with water. To 1,2,3 and 4 ml of the diluted standard add 5,1, 4,1, 3,1, 2,1 ml of the colour stabilizer, mix thoroughly and read against a water blank at 550 nm. The above standards correspond to alkaline phosphatase activities of 25, 50, 75 and 100 Units per litre under the conditions of the assay.

   The standard curve obtained with the Gilford Stasar III (Figure I), plotting the mean absorbance reading for ten repeated preparations of each standard, is effectively a straight line passing through the origin. It is therefore possible to convert test readings to activity units directly by means of a factor.

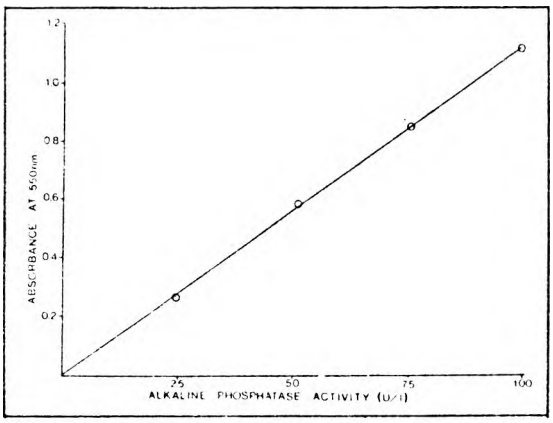

Figure. 1. Phenolphthalein Standard Curve.

5) **Procedure.** Place 0,2 ml of the working substrate in a small glass test tube (12 x 75 mm is suitable) and warm to 37 °C in a water bath. Add 20 µl serum, vortex mix briefly and incubate at 37 °C for exactly 20 minutes. At the end of the incubation add 1,0 ml of colour stabilizer, vortex mix and read the absorbance at 550 nm against a reagent blank set up without serum. Alkaline phosphatase activity is read from the standard curve or calculated by means of the conversion factor, as indicated above.

METHOD EVALUATION

a) **Within Batch Precision.**
This was measured by 20 replicate estimations of Versatol quality control serum, at three different levels:-

1)	Manufacturer's Value	28 U/litre
	Mean	25,62 U/litre
	S.D.	0,51 U/litre
	C.V.	1,98 %
2)	Manufacturer's Value	65 U/litre
	Mean	61,66 U/litre
	S.D.	0,77 U/litre
	C.V.	1,25 %
2)	Manufacturer's Value	102 U/litre
	Mean	95,35 U/litre
	S.D.	1,36 U/litre
	C.V.	1,42 %

b) **Between Batch Precision and Correlation With Routine Automated Method On Patients' Specimens.**

Twenty patients' specimens which had been analysed routinely for alkaline phosphatase on the Technicon SMA 12/60 were analysed twice in separate batch runs by the manual method with the result presented in Table I.

The 12/60 procedure utilized p-nitrophenyl phosphate as substrate and has quoted reference value range of 30-100 U/litre. The reference range for the phenolphthalein method is given by Babson et al as 9-35 U/litre.

The following statistical data can be derived from the results given in Table I:-

Alkaline Phosphatase U/litre

Specimen	SMA 12/60	Manual Method	Rounded mean
1	70	24,3	23,8	24
2	57	21,6	21,3	21
3	75	25,1	24,7	25
4	96	37,1	36,4	37*
5	75	27,1	26,3	27
6	49	16,2	15,7	16
7	215*	64,5	63,3	64*
8	55	20,1	19,3	20
9	155*	37,7	36,8	37*
10	156*	46,5	45,1	46*
11	114*	38,9	38,3	39*
12	77	25,8	25,9	26
13	85	29,7	29,6	30
14	101*	31,5	31,7	32
15	81	28,6	28,4	29
16	119*	38,2	39,0	39*
17	61	20,4	20,3	20
18	272*	80,6	78,0	79*
19	68	24,9	25,2	25
20	87	28,6	28,7	29

* result above upper limit of quoted "normal range"

TABLE I Alkaline Phosphatase values on patients' specimens analysed by the manual micro-method and by a routine automated method.

Standard deviation from difference of duplicates = 0,88 U/litre.

Between batch C.V. at mean level assayed (33,1 U/litre) = 2,65%

Correlation coefficient: Automated method (x) / Manual method (y) = 0,993
Regression equation: Y = 0,273 x + 5,6 U/litre

DISCUSSION

Using the published reference values, the patients' results in Table I can be classified as 'normal' or 'raised' for each of the methods. Of the 20 specimens, 12 were found normal and 6 raised by both methods. Discrepancy occurred only in two cases (specimens 4 and 14) both of which were close to the borderline of the reference range.

The modified phosphastrate procedure here presented reduces the reagent cost per test to just over one cent, which is approximately 5 % of the cost per test of the standard kit method.

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APPENDIX 3

SAIMR QUALITY CONTROL PROGRAMME

Kit for period April-July, 1980

Contents:

Four vials of lyophilised control serum labelled A, B, C, D.
Set of 6 results forms (2 spare).

Instructions:

1. Return dates. Completed results forms are to be returned on the following dates:-

Specimen A	:	Friday 11th April
" B	:	" 9th May
" C	:	" 13th June
" D	:	" 11th July

2. Re-constitution

i. Shake vial to break up lyophilised cake.

ii. Remove metal tear-off seal and rubber stopper.

iii. Using a volumetric pipette, add exactly 10,0 ml distilled water to the vial containing the lyophilised material.

iv. Replace the stopper, and swirl gently to mix. Allow approximately 30 minutes for complete reconstitution or until material is in solution.

v. After complete reconstitution, mix by inversion several times just prior to use to ensure homogeneity.

vi. Pour the reconstituted product directly into sample cups or tubes according to established system operating and laboratory protocols.

vii. Store unused reconstituted material in a tightly stoppered vial at a temperature between $2^{o}C$ and $8^{o}C$ until additional samples are required. Immediately stopper the vial after the additional samples are poured.

viii. Prior to reuse, mix contents thoroughly by inversion, and allow sufficient time (approximately 30 minutes) for the material to reach room temperature.

Storage conditions

Prior to reconstitution, the product should be stored at a temperature between $2^{o}C$ and $8^{o}C$. After reconstitution, it should be stored at a temperature between $2^{o}C$ and $8^{o}C$ when not in use.

Stability

Reconstituted: When prepared and stored as directed, the reconstituted product is stable for at least 24 hours.

Lyophilized: When stored as directed, the lyophilised product is stable until the expiration date that is printed on the label.

3. Analyses

Carry out the analyses by the methods as indicated for your laboratory in the "methods questionnaire" previously returned. Fill in the results where indicated on the left side of the results form, leaving the right hand columns blank.

4. Return address

Biochemical Methods and Equipment Unit,
Room 40,
SAIMR,
P.O. Box 1038,
Johannesburg. 2000

5. Following computer analysis, copies of the results forms will be returned to the individual laboratories showing the overall means, standard deviations and individual laboratory bias.

R.J. GEORGES.
BIOCHEMICAL METHODS AND EQUIPMENT UNIT.

10th March 1980.
/npw.

APPENDIX 4

SAIMR QUALITY CONTROL PROGRAMME RESULTS FORM

Laboratory ..... BARAGWANATH.

Specimen ..... "D"

Date 11.07.80

Test	Result	Unit	Bias (No. of SD + OR-)
Sodium	150	mmol/l	0.65
Potassium	3.0	mmol/l	−0.12
Chloride	115	mmol/l	0.93
Bicarbonate (CO_2)	8	mmol/l	−0.63
Urea	12.8	mmol/l	0.39
Creatinine	376	µmol/l	0.58
Total protein	78.6	g/l	1.10
Albumin	38.4	g/l	0.69
Calcium	2.90	mmol/l	0.77
Inorganic phosphate	2.24	mmol/l	−0.16
Cholesterol	4.94	mmol/l	−0.37
Glucose	9.7	mmol/l	−0.37
Uric acid	0.579	mmol/l	0.78
Total bilirubin	79	µmol/l	−1.01
Alkaline phosphatase	289	Units/l	—
	Temperature 37 °C		
Aspartate transaminse (SGOT)	163	Units/l	—
	Temperature 37 °C		

APPENDIX 5

LOCAL SUPPLIERS OF EQUIPMENT AND MATERIALS

EQUIPMENT

Harleco Micro CO_2 Analyser.	AHSC/South Africa (Pty) Ltd., Johannesburg.
Radiometer CMT-10 Chloride Titrator.	Medical Distributors (Pty) Ltd., Johannesburg.
IL 343 Flame Photometer.	Instrumentation Laboratory South Africa (Pty) Ltd., Johannesburg.
Gilford 300-T Spectro-photometer.	AHSC/South Africa (Pty) Ltd., Johannesburg.
Leitz Model M Photrometer.	Wild and Leitz RSA (Pty) Ltd., Johannesburg.
Oxford Samplers, Pipettors and Dilutor.	Beckman Instruments (Pty) Ltd., Johannesburg.
SMI Micro/Pettor.	Abbott Laboratories SA (Pty) Ltd., Johannesburg.
Clay-Adams Selectapette.	AHSC/South Africa (Pty) Ltd., Johannesburg.
Sartorius 1205 Micro-balance.	Optical Instruments (Pty) Ltd., Johannesburg.
Packard 300-sample Auto-Gamma Counter.	Packard Instrument (Pty) Ltd., Johannesburg.
Venoject blood collecting tubes.	Comopharm (Pty) Ltd., Johannesburg.
Mediconics Chemputer-100.	Warner Pharmaceuticals, Johannesburg.

DIAGNOSTIC KITS, REAGENTS, STANDARDS AND QC MATERIALS

Boehringer Mannheim (SA) (Pty) Ltd., Johannesburg.

Glucose kit (GOD-PAP), No.166391.

Cholesterol kit (CHOD-PAP), No.231347.

"Preciset" cholesterol standards, No.125512.

Clinical Sciences and Manufacturing Laboratories (Pty) Ltd., Johannesburg.

Urea kit "Ureaxact, No.K-UR01.

Calcium kit "Calxact", No.K-CA01.

Lactate-buffered BCG reagent.

Saturated picric acid solution.

BUN acid reagent, No.T01-0612.

BUN colour reagent, No.T01-0401.

Merck Chemicals (Pty) Ltd., Johannesburg.

Evans Blue, No.3169.

0,1 mol/l sodium chloride ("Titrisol").

1,0 mol/l sodium hydroxide ("Titrisol").

Roche Products (Pty) Ltd., Johannesburg.

Diagnostica Cholesterol kit, No.1005.

Technicon Autoanalyser (Pty) Ltd., Johannesburg.

Technicon TQC Chemistry Controls.

Technicon Multi-System Controls.

Technicon SMA Calibrating Serum.

Warner Pharmaceuticals (General Diagnostics Division), Johannesburg.

"Phosphastrate Alkaline" substrate, No.2302.

Calibrate Automated Lock-In Serum, No.1178270.

Wellcome (Pty) Ltd., Kempton Park, Transvaal.

Wellcomtrol and Wellcomtrol QCP control sera.

Worthington Diagnostics (through AHSC/South Africa (Pty) Ltd., Johannesburg.

"Statzyme GOT", No.27940.