LCM 10 (*Supplement—South African Journal of Laboratory and Clinical Medicine*)

THE EVALUATION OF A DISCRETE AUTOMATIC ANALYSIS SYSTEM*

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SUMMARY

An evaluation of an analysis system (Analmatic)† for the performance of routine clinical chemical analyses is reported. Performance data are presented which show that the system generally worked satisfactorily in carrying out its functions of dispensing and diluting, performing colorimetric and flame photometric measurements and recording results.

Various points of comparison with the Autoanalyser‡ continuous-flow system are discussed. It is considered that the Analmatic system, through its lack of versatility and lesser degree of continuously mechanized performance, is at present only of limited usefulness in the routine clinical laboratory.

The testing and evaluation of new scientific equipment has in recent years become another of the important tasks of the clinical chemist working in a routine chemical pathology laboratory. The development of this new scientific activity has resulted from the following circumstances:

1. Twenty years ago the commonly used methods of quantitative clinical chemistry were based on the classical techniques of gravimetric, volumetric and gasometric analysis, and the then relatively new technique of photoelectric colorimetry. The introduction since that time of routine methods based on flame photometry, ultraviolet spectrophotometry, fluorimetry, etc., and the greatly increased use of instrumental methods of analysis, have given the modern clinical laboratory a completely new look.

2. A natural consequence of the development of instrumental analysis has been an enormous growth in the number of instrument manufacturers and the range of their products. The scientist seeking an instrument for some particular purpose is now frequently faced with a bewildering number from which to make a selection. The increased complexity of much of this equipment can make it impossible for the would-be purchaser's queries to be answered in full by the manufacturer's specifications. Furthermore, the performance claimed, and even the price, of rival instruments are often closely similar.

3. Finally, but in many cases most importantly, the high cost of sophisticated modern instrumentation makes it essential to spend wisely; no laboratory head can afford to chance the allocation of a sum of money, which may represent a substantial portion of his annual budget, to the purchase of an instrument which turns out to be unsatisfactory in use.

Among the most complex and expensive of instruments for the clinical chemistry laboratory are the modern automatic devices designed to carry out mechanically the routine chemical estimations, such as blood urea, sugar, proteins and electrolytes, previously performed batchwise by manual methods. Until recently, however, the circumstances of section 2 above did not operate, inasmuch as only one type of automated equipment had achieved widespread use. This system was based on the 'continuous flow' principle devised by Skeggs[1] in 1957 and made available commercially by the Technicon Corporation. In this system specimens are taken up and injected at regular intervals into a liquid stream, and all the resultant dilutions from successive samples, together with reagents, flow in the same pathway throughout the entire analytical process.

An alternative automatic system involves the mechanization of the various steps of manual analysis : sampling, dilution, transfer, reagent addition, etc., while keeping the solutions derived from successive samples in separate vessels through all stages up to the final physical measurement. Within the last 4 - 5 years about 20 such 'discrete' analytical systems have appeared on the market (for a general review, see Northam.[2]) The evaluation of these machines is now proceeding actively in several centres, including a number in South Africa.

Through the courtesy of the manufacturers† we have recently carried out a limited evaluation of the Analmatic discrete analysis system. The present study presents some of the factual data acquired during this evaluation, together with a consideration of the relative advantages and disadvantages of the continuous-flow and discrete systems.

OPERATION OF THE 'ANALMATIC' SYSTEM

A schematic representation of the various components is shown in Fig. 1. The following description is necessarily brief and should be supplemented by reference to the manufacturer's literature.

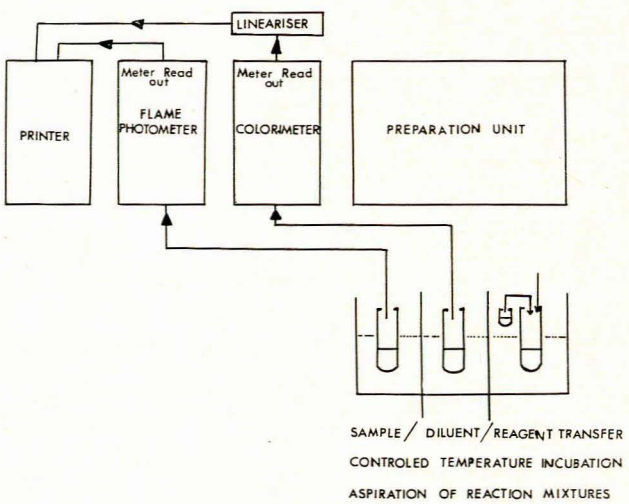

Fig. 1. Modular assembly of the Analmatic system.

The preparation unit can accommodate up to 100 tubes in 10 rows of 10. Through the operation of mechanically driven syringes, samples are automatically taken up from sample cups and delivered into their individual reaction tubes together with diluent and any necessary reagents. The tubes can be immersed in circulating water from a

*Date received: 22 October 1970.
†Baird and Tatlock (S.A.) (Pty) Ltd, Johannesburg.
‡Technicon Autoanalyser (Pty) Ltd, Johannesburg.

TABLE I. FACTORS WHICH COULD GIVE RISE TO FAULTY PERFORMANCE IN THE DISCRETE CHEMICAL ANALYSIS SYSTEM

Function or system undergoing fault	*Cause of fault*
Uptake and delivery of sample, diluent and reagents	1. Insufficient sample in cup. 2. Incorrect alignment of sampling probes and delivery tips in relation to tubes. 3. Faulty syringe action or valve control. 4. Faulty movement of dipper head or of sample carriage. 5. Blockage of tubing.
Aspiration of reaction mixture into colorimeter or flame photometer	6. Suction set too high or too low (also 2, 4, and 5 above)
Stability and reproducibility of instrumental readings	7. Drift of printer output on O-volt or 1-volt calibrations. 8. Drift in colorimeter photocell response. 9. Drift in lithium, sodium or potassium photocell response.
Maintenance of successive samples and reaction mixtures free from mutual interaction	10. Contamination of specimen by carry-over from preceding cup. 11. Contamination of specimen by diluent. 12. Carry-over in the colorimeter flow-cell. 13. Carry-over in the flame photometer.

heating bath if controlled-temperature incubation is required.

For colorimetric or flame photometric determinations the reaction mixture is aspirated into the flow-cell of the colorimeter, or into the gas stream of the flame photometer. The output of the photocell of either instrument can be read directly or else fed to a digital printer which may be calibrated to give a print-out in concentration units.

SCOPE OF INVESTIGATIONS

It was decided at the outset to concentrate on an evaluation of various individual functioning components rather than attempt to assess 'accuracy' by extensive analysis of control serum or of patients' specimens in parallel with the department's routine methods.

Some factors which might give rise to unsatisfactory performance by a discrete system of this type are shown in Table I. Our scheme or evaluation was concerned with factors 7, 8, 9, 10, 12 and 13. Methods used to assess contamination and carry-over were based on those proposed by Broughton et al.[3] In addition, an assessment of over-all performance was made by measuring the precision in preparing and measuring a simple dilution, and in carrying out the estimation of total protein, sodium and potassium and alkaline phosphatase on pooled human and quality control sera.

METHODS AND RESULTS

Instrumental Drift

Following the recommended 15-minute warm-up period the zero drift of the printer and colorimeter were both found to be negligible. The printer response to a 1-volt signal and the colorimeter reading of optical density of a coloured solution both showed a slight upward drift with time. The cumulative drift of the two instruments during the period from 20 to 40 minutes after switching on was between 1% and 1·4%. This 20-minute period would allow for colorimetric reading on a full batch of 100 specimens.

The reading of the lithium photocell of the flame photometer showed a rather more serious downward drift with time. Since the reading of sodium or potassium depends on the 'balance' of the appropriate photocell against the lithium photocell, the sodium and potassium readings both showed an upward drift with time, amounting to 5 - 8% during the 20-minute period from 30 to 50 minutes after switching on the flame photometer.

Cup-to-Cup Carry-over in the Preparation Unit

With a fixed quantity of serum (0·5 ml) in each cup the carry-over found was as follows:

Total protein method	0·15%*
Sodium/potassium method	0·05%*

Carry-over in the Colorimeter Flow-Cell

On aspirating water immediately after a solution of high optical density (potassium permanganate) the maximum carry-over found was 0·125%, or 1 part in 800.

Sample Interaction in the Flame Photometer

On aspirating a 'high' test solution after a lower one it was found that a depression in sodium and potassium readings occurred. Conversely, carry-over from a 'high' test solution to a low one caused an augmented reading. The extent of this interaction ranged from 8 - 14% of the true concentration difference between the two solutions. A round figure of 10% for this interaction meant, for example, that a 'true' value of 120 mEq/litre following immediately after a 'true' value of 150 mEq/litre would read out as $120 + (150 - 120) \times \frac{10}{100} = 123$ mEq/litre.

Reproducibility of Diluting Function

Dilution of potassium permanganate solution. A solution of potassium permanganate was prepared which on manual 1:51 dilution with water gave an optical density at 535 nm around 0·40. This solution was automatically diluted and sampled through the colorimeter. The optical density values at 535 nm against a water blank were printed out.

Results:

Number of estimations	24
Mean print-out value	418
Standard deviation	10·4
Coefficient of variation	2·5%

Dilution of methylene blue solution. A solution of methylene blue was prepared which on manual 1:51 dilution gave an optical density against water at 490 nm of around 0·60. This solution was automatically diluted and sampled through the colorimeter.

*Mean of 5 estimations.

Results:

Number of estimations	42
Mean print-out value	614
Standard deviation	14·8
Coefficient of variation	2·4%

Dilution of a protein solution. A solution containing 5 g/100 ml ^{131}I-labelled protein was automatically diluted. The dilutions were measured for radioactivity in a well-type scintillation counter. Counts were made for 100-second periods, the mean background (639) was 3·5% of the mean total corrected count.

Results:

Number of estimations	15
Mean corrected count	18 120
Standard deviation	359
Coefficient of variation	2·0%

Over-all Reproducibility of Total Protein Estimation (biuret colorimetric method)

Number of estimations	47
Mean value	6·95 g/100 ml
Standard deviation	0·146 g/100 ml
Coefficient of variation	2·1%

Over-all Reproducibility of Sodium Estimation

Number of estimations	20
Mean value	136·7 mEq/litre
Standard deviation	2·2 mEq/litre
Coefficient of variation	1·6%

Over-all Reproducibility of Serum Alkaline Phosphatase Estimation (hydrolysis of sodium p-nitrophenyl phosphate)

Number of estimations	20
Mean value	27·8 KA units
Standard deviation	0·94 KA units
Coefficient of variation	3·4%

Failures in Instrument Performance

During the 1-month period of operation the system was at no time put out of action by mechanical failure. Minor failures in performance which did occur were as follows:

Preparation unit. Some of the syringes, notably the one dispensing biuret reagent in the protein method, 'seized' on standing overnight and had to be released by manual pressure. The stop-bar controlling the stopping position of the sampling head became displaced on 3 occasions, resulting in premature stopping. The reaction tray once failed to advance at the end of the first row of tubes, resulting in a whole row being sampled twice.

Colorimeter. The colorimeter flow-through system was found unable to deal with a non-aqueous solution (glacial acetic acid). Carry-over was excessive with this liquid and the nipple connections to the flow-cell became detached after a short period of operation.

Printer. On 4 occasions the printer operated spontaneously, possibly due to a mains 'surge'. During one run of electrolyte determinations the printer locked on 000 and failed to print out 3 potassium results.

DISCUSSION

With the exceptions noted above the Analmatic system gave a trouble-free mechanical performance. Our experimental findings show that this equipment is able to perform the technical functions of metering and transferring fixed quantities of sample and reagents, and the measurement and recording of optical density, with a high degree of precision.

The upward drift in sodium and potassium readings, evidently caused by a fading in the balancing lithium photocell, was an unsatisfactory feature of the particular model tested. The fault could be overcome, at some expense of the operator's time and in work output, by recalibrating on standards interspaced frequently within a batch of tests.

When considering the introduction of some form of automation into the clinical chemistry laboratory, it is necessary to define carefully the requirements and decide on whichever of the available systems meets these most satisfactorily. The relative advantages and disadvantages of the continuous-flow and discrete systems have been reviewed recently by a number of authors,[4,5] and therefore only certain points of comparison will be considered here.

The most obvious advantage of the Analmatic system over the Autoanalyser is the greater 'throughput' of some (although not all) analyses. The maintenance of reaction mixtures in discrete compartments allows them to be presented to the colorimeter at the rate of 1 sample every 12 seconds, or 300 samples per hour, compared with a rate of about 60 per hour for the standard single-channel Autoanalyser. Colour measurement under steady-state conditions greatly simplifies the processes of computation and print-out. However, it must be borne in mind that the rate of 300 operations per hour applies only to any individual step, such as sampling, reagent addition, colour measurement, etc. If these steps require to be carried out in a timed sequence, rather than simultaneously, the actual rate of throughput will be reduced. In this connection also, the limitation of the Analmatic system to a single working channel for most analyses must be regarded as a disadvantage compared with the Autoanalyser's capacity for multi-channel operation.

The Analmatic is a discontinuous system, requiring operator intervention at various stages in the course of a run of analyses. Further operator involvement results from the necessity of providing the individual reaction tubes. The most serious cause of discontinuity is the necessity for the tubes to be transferred manually to and from a centrifuge when preparing a protein-free filtrate. The system of deproteinization by dialysis used in the Autoanalyser is a major advantage of the continuous-flow system. However, active research is taking place in the development of new methodologies which do not involve deproteinization, and the Analmatic system, through its ability to make simultaneous samplings of a test and 'serum blank', is well designed to make use of such methods.

An important advantage of the Autoanalyser is the confidence provided by the chart record of analytical performance. The baseline, drawn continuously by the recorder between sets of specimens, is a very useful indicator of drift, while inspection of the test tracings can often detect and even locate analytical faults.

At the present time the Autoanalyser has a considerable lead over all other systems in its versatility with

regard to the number of different analytical devices used and different estimations undertaken. A number of standard Autoanalyser methods, such as the ferricyanide method for glucose and the diacetyl method for urea have proved highly successful in the continuous-flow system, but less satisfactory in discrete systems. The Autoanalyser method for bicarbonate, involving the decolorization of a buffered phenolphthalein solution, can only operate in a 'closed' continuous-flow system.

Present and Future Developments

Discrete methods of automatic biochemical analysis are still in the early stages of development and utilization. Realization of the full potential of this approach lies in the future. At the present time the usefulness of a system such as the Analmatic in the routine performance of laboratory tests is severely limited. It would appear likely, however, that future mechanical modifications and developments in the underlying chemistry of analytical methods will ultimately allow a place for both types of automatic apparatus in the laboratory.

Many of the mechanical devices which are integrated in the complex discrete analyser can be used individually for purposes of limited-function automation[6] or work simplification. The incorporation of such devices into the routine laboratory would be highly rewarding for the clinical chemist at the present time.

We wish to thank Mr David Knight of Baird and Tatlock (South Africa) (Pty) Ltd, for his unstinting technical co-operation; and the Director of the South African Institute for Medical Research for permission to carry out and publish this work.

REFERENCES

1. Skeggs, L. T. (1957): Amer. J. Clin. Path., **28**, 311.
2. Nørtham, B. E. (1969): J. Clin. Path., **22**, suppl. **3**, 42.
3. Broughton, P. M. G., Buttolph, M. A., Gowenlock, A. H., Neil, D. W., and Skentelbery, R. G. (1969): *Ibid.*, **22**, 278.
4. Broughton, P. M. G. (1969): *A.C.B. Technical Bulletin No. 16*. London: Association of Clinical Biochemists.
5. Mitchell, F. L. (1969): J. Clin. Path., **22**, suppl. **3**, 51.
6. Hardy, S. M. (1968): Z. Chem., **243**, 713.