A double sampler unit for the AutoAnalyzer

D. EVERARD and G. SEYMOUR *From the Department of Chemical Pathology, St. Mary's Hospital, London*

It has been possible to modify the sampler module of the AutoAnalyzer to enable laboratories possessing additional recorder and colorimeter units to analyse two sets of specimens simultaneously.

Basically the device consists of a circular plastic channel of rectangular cross-section which fits on top of the normal sample plate and carries a second ring of cups (Fig. 1). The external diameter is 18.5 cm., internal diameter 14.8 cm., width of channel 1.4 cm., thickness of plastic 0.2 cm., height of internal wall 1.8 cm., and height of external wall 2 cm.

FIG. 1

The channel is secured in position by means of three holes bored in the base in such a way as to fit over the three small screws projecting from the normal sample plate. A second sample crook made from $\frac{1}{8}$ in. nickel tubing and braised to the normal one, dips into cups placed in the channel. Plastic cups $\frac{1}{8}$ in. in diameter by 1 in. long obtainable from Camlab Ltd. are used and are the same size as those sold by this firm as disposable blood sugar containers. Thus if these tubes are used for collecting blood from the patient, they may be placed directly on the modified sample plate without transferring.

This additional sample holder, together with an extra recorder and colorimeter unit, produces in effect two

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An inexpensive method for the estimation of true glucose in blood and other fluids by the AutoAnalyzer

GEORGE DISCOMBE *From the Central Middlesex Hospital, London*

An enzymic micro-method for the estimation of glucose in blood and other fluids by the AutoAnalyzer is described. It uses no more than 0.16 ml. blood and therefore can be used for capillary blood samples from the finger or ear; it is not affected by fluoride in concentrations up to molar; and the reagents cost only about 2d. per blood sample.

Huggett and Nixon (1957) described a method for the estimation of true glucose in blood which was insensitive to the presence of 10 mg. sodium fluoride per millilitre blood. They used a reagent which contained glucose oxidase, horseradish peroxidase, and *o*-dianisidine. Hill and Kessler (1961) adapted this for the AutoAnalyzer, but in doing so increased the concentration of peroxidase fortyfold, presumably to ensure that, however much fluoride were added to the blood, there would still be an excess of enzyme. This increased the cost of the reagent to an average of 9d. per blood sample, and this high cost, together with the large sample required (0.42 ml.), made the method unsuitable for most routine laboratories. The more economical method of Wincey and Marks (1961) uses formaldehyde to preserve blood samples: as it requires predilution of the blood it is less convenient.

In the absence of fluoride the concentration of peroxidase required in the Hill and Kessler method can be reduced about twentyfold. Modifications intended to remove fluoride were therefore tried, and it was found possible to use a reagent containing one fifteenth of the original concentration of peroxidase and one half to one

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A double sampler unit for the AutoAnalyzer—concluded

AutoAnalyzers, since using 15 line end-pieces any two manifolds may be combined, the dialyser has room for two units, and two heating coils may, if necessary, be fitted in one heating bath.

Using this device the following estimations have been successfully combined:—Alkaline phosphatase and bilirubin, carbon dioxide and sugar, and uric acid and inorganic phosphorus.

Double runs of the same analyses are also possible; thus with two sugar manifolds combined, it is possible to analyse 80 samples per hour.

third that of the glucose oxidase without significant reduction in sensitivity. If a double dialyser be used, the new micro tubing enables the sample volume to be reduced to 0.16 ml., and although the sensitivity is as high as 14 to 19 transmission lines for a 100 mg. standard, the cost of reagent is reduced to about 2d. per sample. It is possible to make estimations over the range 20 to 2,000 mg. % without any alteration in procedure, but above 700 mg. % the precision is reduced greatly.

PRINCIPLE

Fluoride is precipitated as magnesium fluoride before dialysis and the phosphate buffer originally used is replaced by a *tris*-maleic acid-caustic soda buffer (Gomori, 1946, 1955) to avoid precipitating the magnesium.

REAGENTS

DILUENT Magnesium sulphate heptahydrate A.R., 40 g. per litre.

RECIPIENT Anhydrous sodium sulphate A.R., 16.1 g. per litre, containing 0.5% Triton X-100 (Rohm and Haas) or surface active agent A.P. 14 (Imperial Chemical Industries).

CHROMOGEN *o*-Dianisidine (Hopkin and Williams), 500 mg., is dissolved in 100 ml. hot ethanol and diluted with water, and 250 ml. glycerol (A.R.) is added shaking continuously: the mixture is diluted with water to 1,000 ml. and kept in a brown glass bottle. It is filtered through a no. 1 Whatman paper immediately before use.

BUFFER For the stock solution 19.6 g. maleic anhydride is dissolved in warm water, and 24.2 g. *tris*-(hydroxy-

methyl)-amino-methane dissolved in it. The mixture is cooled to room temperature and diluted to 1,000 ml. with water.

For the working buffer 250 ml. stock solution is placed in a 1-litre flask, normal sodium hydroxide is added (26 ml. for pH6 and 48 ml. for pH7), and water is added to the mark.

ENZYME REAGENT Dilute solutions of the enzymes used readily grow moulds and bacteria and their activity is greatly reduced by freezing and thawing. We therefore prepare a concentrated solution which keeps for months at +4°C. and dilute it shortly before use. This concentrated solution is prepared by placing 125 ml. glucose oxidase solution (Fermcozyme 653A containing 750 units per ml.) and 100 mg. horseradish peroxidase of $R_z > 1$ in a 500 ml. flask, dissolving in buffer and diluting to the mark. For use this is diluted 1 in 15 with buffer, and then gives a sensitivity of about 19 transmission lines for a 100 mg./100 ml. standard. The sensitivity can be varied over a moderate range by altering the quantity of glucose oxidase used. If it is necessary to use peroxidase of less activity than that specified, the quantity must be increased, e.g. for peroxidase of $R_z = 0.6$, it would be desirable to use 166 mg. to each 500 ml.

PROCEDURE The analyses are performed using a double dialyser and the manifold shown in the flow chart, the sampler module being run at 60 samples an hour and alternate spaces being left empty: a water wash is inserted after the high standards. The calibration curve is set up for each series using standards of 25, 50, 100, 150, 200, 250, 300, 500 mg./100 ml. and if thought desirable, 700, 1,000, and even 2,000 mg./100 ml., though above 700 mg./100 ml. the precision of the method is greatly reduced. The calibration curve is linear to about 150 mg./100 ml.

FIG. 1. Flow diagram for micro-glucose estimation on the AutoAnalyzer using glucose oxidase and peroxidase.

TABLE I

REPRODUCIBILITY OF MACRO METHOD USING HILL AND KESSLER MANIFOLD FLUORIDE CONTENT MOLAR¹

<i>Series A</i>					
Mean estimated glucose concentration (mg./100 ml.)	52.4	110.3	217.5	328.7	537.7
Coefficient of variation (%)	1.9	1.8	1.86	4.4	2.2
<i>Series B</i>					
Mean estimated glucose concentration (mg./100 ml.)	56.3	114.0	221.7	334.1	532.5
Coefficient of variation (%)	0.9	7.8	1.75	1.5	0.95

¹One analysis using each of 12 different enzyme and buffer combinations, at pH between 6 and 7.

TABLE II

REPRODUCIBILITY OF MICRO METHOD¹

Standard (mg./100 ml.)	25	50	100	150	200	250	300	500
Observed (mean of 5 estimations)	25.4	52.2	103.8	151.2	200.4	249.4	299.6	511
Coefficient of variation (%)	6.7	3.6	5.2	0.6	3.4	3.4	3.4	2.3

¹Standards in benzoic acid compared with standards containing up to M fluoride

but above that level curvature is slight up to 300 mg./100 ml. The colorimeter can be operated at any wavelength from 430 to 480 m μ , the optimum being 450 m μ . After each batch the dialyser is washed with 0.1% sodium azide or 0.1 N sulphuric acid to prevent the growth of moulds which may digest the cellulose and cause rapid perforation of the dialysing membrane.

RESULTS

Aqueous solutions of glucose containing M potassium fluoride were kept and repeatedly analysed during two fortnightly periods two months apart. The 'macro' manifold of Hill and Kessler was used, with the reagents described here, except that the enzyme reagent¹ varied in pH and concentration, 12 different combinations being studied. The results obtained are given in Table I.

When micro-tubing became available and the new manifold was introduced, it was found that the carry-over from one sample to the next was about 3% and a water wash was inserted after the high standards. To assess the effect of fluoride, potassium fluoride solutions containing 8, 20, and 60 mg. of the salt were evaporated in sample cups, 1 ml. volumes of standard glucose solutions were added to each, and these three, together with two untreated standards, were analysed, the calibration curve being made from the same standard solution run previously in the same batch. The results are shown in Table II. The reproducibility of the two procedures was further checked by repeated analyses on three blood samples, two by the macro and one by the micro method (Table III).

Recoveries were checked by adding 1 ml. of 200 or 250 mg. % glucose standard to 5 ml. blood preserved with fluoride, and performing repeated analyses by the micro

¹The enzyme preparations were obtained from Messrs. Hughes and Hughes (Enzymes) Ltd., 10 Stratton Street, London, W.1.

TABLE III

REPRODUCIBILITY OF METHOD ON BLOOD SAMPLES

	Macro	Micro
Blood glucose (mean of 9 analyses) (mg./100 ml.)	72.9	157.5
Coefficient of variation (%)	2.7	1.9

TABLE IV

RECOVERY EXPERIMENTS USING MICRO METHOD¹

Expected (mg.)	x	x + 33.3	x + 41.66
Observed (mean of 11 estimations)	63.63	96.625	106.27
Difference	—	32.989	42.64
Deviation from expectation (mg.)	—	0.34	1.02
Coefficient of variation (%)	2.15	1.5	1.93

¹Fluoride content 2.4 mg./ml. blood

method; as a control 1 ml. of saline was added to 5 ml. blood. The results are in Table IV.

The method has been in routine use for several months. The chief difficulties have been in identifying blood samples in the sample cups and in obtaining enough blood (about 0.3 ml.) from a skin prick on a cold day.

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REFERENCES

- Gomori, G. (1946). *Proc. Soc. exp. Biol. (N.Y.)*, **62**, 33.
 — (1955). In *Methods in Enzymology*, edited by S. P. Colowick and N. O. Kaplan, vol. I, p. 143. Academic Press, New York.
 Hill, J. B., and Kessler, G. (1961). *J. Lab. clin. Med.*, **57**, 970.
 Huggett, A. St. G., and Nixon, D. A. (1957). *Lancet*, **2**, 368.
 Winey, C., and Marks, V. (1961). *J. clin. Path.*, **14**, 558.