Rapid Automated Micro Screening for Diabetes

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AUTOMATED METHODS for determination of blood sugar values in diabetes screening are becoming increasingly popular. Equipment and methodology have in the past necessitated use of several milliliters of venous blood. This study was undertaken to develop, with the aid of the AutoAnalyzer (*A*) for determination of blood glucose levels, an automated technique that would result in a rapid, simple, and economical diabetes screening procedure providing a precise measure of blood sugar. Another purpose was to determine the accuracy and feasibility of using the Unopette (*B*) to obtain the blood sample.

The Unopette, a device consisting of a self-filling capillary pipette and a reservoir (fig. 1), was used to simplify the diabetes screening procedure. The pipette is used to obtain the capillary sample, which is then diluted in the plastic reservoir. The entire unit is disposable. Use of the Unopette greatly reduces the amount of time and skill required to obtain the blood sample.

Methods

The Unopette tube fills readily by capillary action with 20 microliters of blood. The tube is then introduced into the reservoir. In this study the reservoir contained 1.3 ml. of a 1 percent sodium fluoride solution. (The constituents of the reservoir were made especially for the study by the manufacturer of the Unopette.)

No unusual precautions were taken in using the Unopette. Care was exercised to see that the capillary tube filled completely, that excess blood was removed from the tip, and that the reservoir was compressed before the capillary tube was introduced into the solution, insuring initial removal of blood from the capillary tube. The tube was then flushed with the diluting fluid by repeated compression of the sides of the reservoir.

The technique in this procedure, schematically diagramed in figure 2, is a modification of the Technicon micro method for glucose determination (*1*). A single heating coil, a single type C membrane, and a 15 mm. tubular flow cell were used. The sampling rate was 60 specimens per hour. A constant-volume device with double-sample crook was used to maintain a steady baseline. The sample cups were kept covered as much as possible to avoid evaporation. The alkaline potassium ferricyanide reagent was diluted more than that in the prescribed Technicon method (*1*) to provide greater sensitivity and allow results to be read with a standard glucose comparator.

The two reagents were potassium cyanide (Technicon AR–16–56), composed of NaCl 9.0 gm., KCN 5.0 gm., and H_2O sufficient to make 1 liter, and alkaline potassium ferricyanide, composed of NaCl 9.0 gm., K3Fe(CN)6 0.125 gm., Na_2CO_3 20.0 gm., and H_2O sufficient to make 1 liter. Technicon reagent AR–124–62

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may be combined with an equal volume of a diluent composed of NaCl 9.0 gm., NA_2CO_3 20.0 gm., and water sufficient to make 1 liter and this combined reagent substituted for the potassium ferricyanide solution.

Sufficient saturated benzoic acid was added to 10 ml. of a stock standard solution to make 1 liter. The stock standard solution consisted of 15.15152 gm. anhydrous dextrose and sufficient saturated benzoic acid (about 4 gm.) to make 1 liter.

A series of working standards was prepared by diluting 5, 10, 15 ml., and so on, of the dilute stock standard to 100 ml. with saturated benzoic acid. Five ml. of dilute stock standard in 100 ml. is equivalent to 50 mg./100 ml. glucose in whole blood which has been diluted through use of the Unopette.

Twenty microliters of sample was used for each determination. This was diluted with 1.3 ml. of a 1 percent sodium fluoride solution to provide a sample of sufficient quantity to be run in the AutoAnalyzer. This dilution was accomplished either with the Unopette or through standard laboratory pipetting procedures. One percent sodium fluoride prevented loss of glucose from the blood samples.

Three types of samples were tested to obtain the required information. First, five known glucose concentrations were constituted in a saturated solution of benzoic acid and distilled water as previously described. The concentrations were then diluted with 1 percent sodium fluoride to equal the dilution obtained with the Unopette (1:66). A single dilution was made for each of the five values. Consecutive runs of each diluted sample were made and the standard deviation was determined (table 1).

Second, a set of studies was conducted on whole, heparinized blood to which sufficient

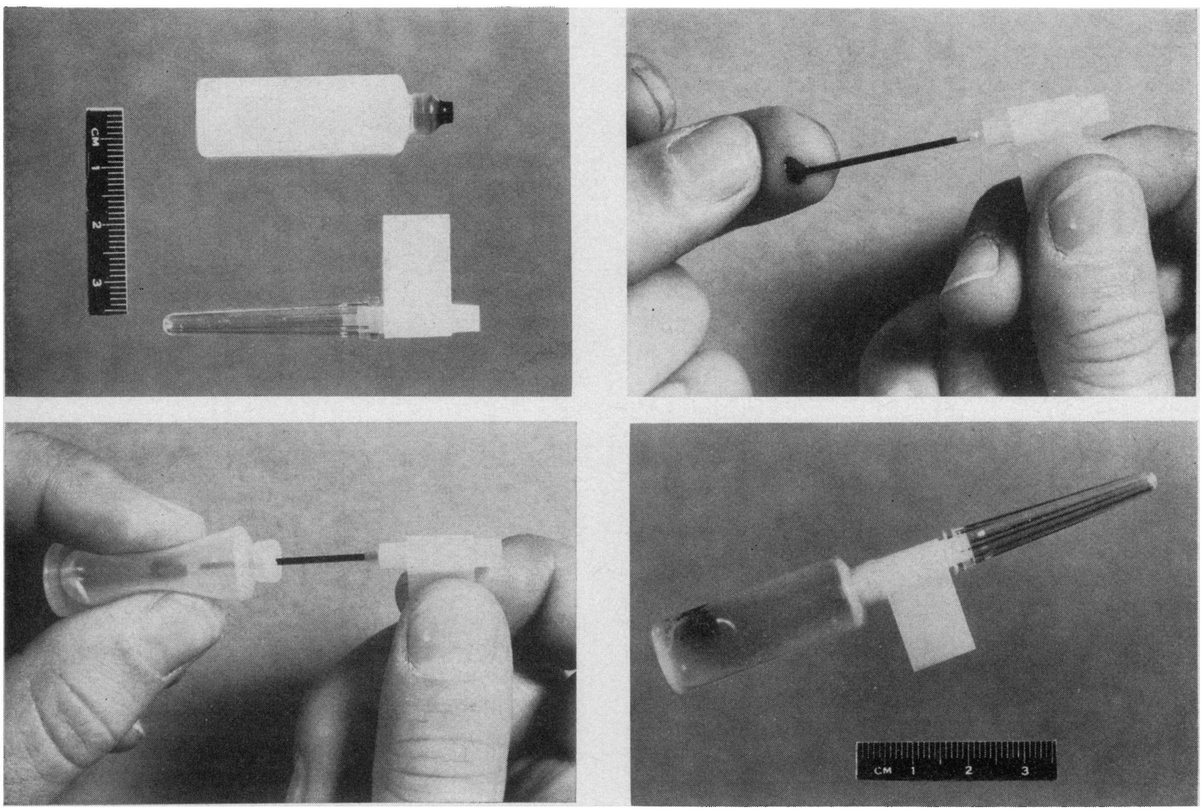
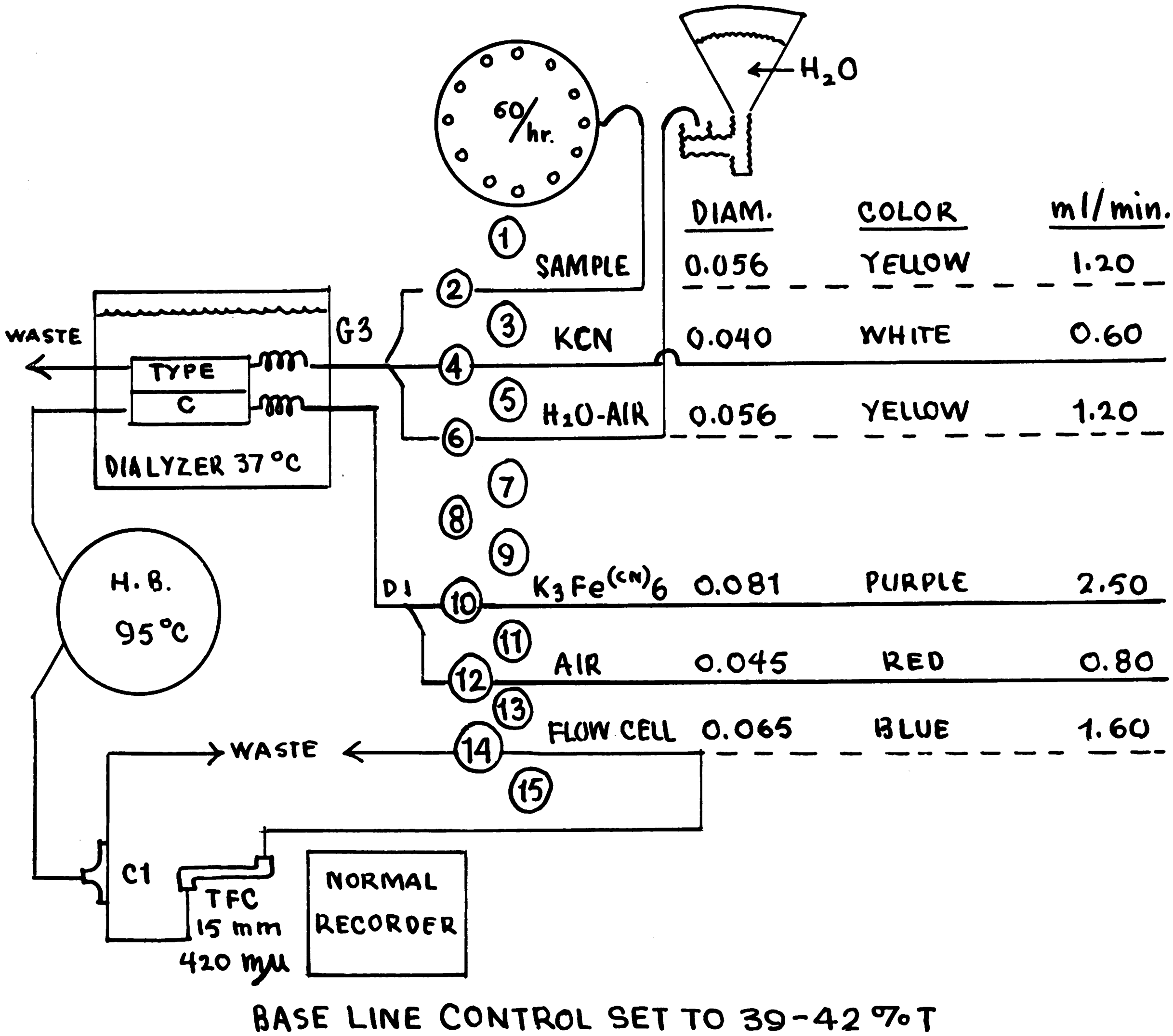


Figure 1. The Unopette. ***Top left***—**sealed reservoir and capillary pipette with guard as supplied by manufacturer.** ***Top right***—**capillary blood sample being collected by 20 microliter self-filling capillary pipette (capillary only partly filled).** ***Bottom left***—**reservoir sides compressed prior to introduction of pipette and sample into reservoir.** ***Bottom right***—**the whole unit capped with the capillary shield and ready for transfer to processing center.**

Figure 2. Schematic for micro glucose determinations using the Unopette and AutoAnalyzer



NOTE: Sample size—20 microliters; diluent—1.3 milliliters of 1 percent NaF; baseline control set to 39–42 percent transmission.

sodium fluoride had been added to make a 1 percent solution. Varying amounts of glucose were then added to four equal portions of the fresh sample to give four different blood glucose values (approximately 50, 127, 175, and 210 mg./100 ml.). The four whole blood samples of varying glucose levels were manually diluted with a 1 percent NaFl solution with Ostwald-Folin pipettes and by standard laboratory procedure to produce a dilution identical to that obtained by the Unopette (samples A, B, C, and D). A single dilution of whole blood was made for each blood sugar value and in sufficient quantity to be used for all tests. Consecutive runs of each of the four diluted blood glucose values were made and the standard deviation computed (table 2).

Third, a series of determinations was made in which samples C (175 mg./100 ml.) and D (310 mg./100 ml.) were run alternately; individual dilutions made with the Unopette replaced the final dilution in the preparation of samples C and D. The higher values of blood sugars were chosen so that small errors in the Unopette operation would become more apparent. Sample C was alternated with its Unopetted equivalent and sample D with its Unopetted equivalent to eliminate avoidable time-related errors from the comparison (table 3).

An additional series of tests was run to determine the effect of the preceding sample on the following sample. A test standard of 125 mg./100 ml. was used, which was obtained with a saturated benzoic acid solution and a weighed amount of glucose. Preceding values of 50, 200, and 300 mg./100 ml. were chosen, using glucose and water standards. The observed values of the test sample were then compared with the expected value and the difference determined (table 4). Ten determinations were made for each of the three preceding values chosen.

Results

Consecutive samples of same value. Table 1 lists standard deviations for consecutive values of glucose in water standards. Table 2 shows the standard deviations for consecutive determinations on diluted whole blood samples obtained from a single, manual dilution of whole blood using Ostwald-Folin pipettes and standard laboratory procedures. The standard deviation for diluted whole blood was significantly greater than that for the glucose in water standard. The single dilution provided sufficient samples for the entire run, thereby eliminating individual pipetting errors.

Table 1. Reproducibility of AutoAnalyzer results in consecutive microglucose determinations on five glucose and water samples of varying glucose concentrations

Glucose in water (mg./100 ml.)	Number of determinations	One standard deviation (mg./100 ml.)
50	48	±1. 45
100	50	±1. 75
150	49	±1. 39
200	48	±1. 85
250	49	±2. 46

Table 2. Reproducibility of AutoAnalyzer results in consecutive microglucose determinations on four blood samples of varying glucose concentrations

Samples of glucose in whole blood	Number of determinations	Mean result	One standard deviation (mg./100 ml.)
A	38	50. 4	±1. 62
B	39	127. 1	±3. 49
C	41	175. 2	±3. 71
D	31	209. 8	±3. 46

Table 3. Comparison of glucose values from individually Unopetted samples with glucose values from samples obtained from a single dilution of the same whole blood sample

Source and type of dilution	Number of determinations	Mean result	One standard deviation (mg./100 ml.)
Whole blood 1:			
Single dilution	36	176. 5	±3. 09
Individually Unopetted	41	175. 2	±3. 71
Whole blood 2:			
Single dilution	31	212. 2	±3. 71
Individually Unopetted	31	209. 8	±3. 46

NOTE: Samples being compared were alternated in the AutoAnalyzer; for example, Unopetted sample—single dilution aliquot—Unopetted sample—single dilution aliquot.

The AutoAnalyzer was carefully adjusted and supervised to obtain maximum reproducibility of results. Careful adjustment of the constant-volume device to insure uninterrupted flow of fluid was found to improve reproducibility. A slight baseline instability was present throughout, which accounts for a portion of the variation.

Accuracy of the Unopette. Design specifications of the 20 microliter Unopette permit a tolerance range of from 0.01966 to 0.02034 ml. for the capillary pipette and a tolerance range of 1.298 to 1.308 ml. for the 1.3 ml. reservoir. Table 3 compares individual Unopette dilutions with a single manual dilution of whole blood. Alternating aliquots of the single manual dilution with Unopette dilutions minimized time-related sources of error in comparing the two. The standard deviation was no greater for the Unopette dilution than for the single manual dilution. The difference in the mean of the Unopette dilutions as contrasted to that of the manual dilutions was of borderline significance, the probables lying between 0.01 and 0.1. Only a single manual dilution was made, and it is likely that any error present was introduced at this time.

Effect of previous sample. Table 4 shows the values obtained when the preceding sample differed from the test sample by significant amounts of glucose. A test sample of 125 mg./100 ml. was used. When the test sample was preceded by a value of 50 mg./100 ml., the observed average value of the test sample was 124.5 mg./100 ml.; when preceded by a value of 200 mg./100 ml., the observed average value of the test sample was 125.5 mg./100 ml.; and when preceded by a value of 300 mg./100 ml., the observed average value of the test sample was 127.1 mg./100 ml. The direction of the error was consistently towards the value of the preceding sample. This error is insignificant for screening purposes.

Discussion

Improved equipment for the AutoAnalyzer has made it possible to use as little as 20 microliters of blood for successful blood glucose determinations. Such advances allow more chemical determinations to be performed on the same sample and also simplify collection.

Our intention in this study was to develop a practical screening method for diabetes which would result in efficient use of the samples obtained. The results do not demonstrate an accuracy equal to that obtained with AutoAnalyzer macro blood sugar determinations run at 40 samples per hour (*2*). This high degree of accuracy, however, is not essential in screening procedures although it can be obtained by micro technique (*3*). In this particular study the more rapid sampling rate of 60 specimens per hour was considered a greater asset than a smaller standard deviation.

Table 4. The effect of varying glucose levels in consecutive samples, expressed in mg./100 ml.

Preceding sample	Test sample of 125		
	Expected	Observed (mean ± 1 standard deviation)	Difference (observed-expected)
50	125	124.5 ± 1.91	−0.5
200	125	125.5 ± 1.69	+0.5
300	125	127.1 ± 1.30	+2.1

NOTE: Glucose and water standards were used and 10 determinations made of each value.

In spite of the inherent accuracy built into the AutoAnalyzer system, careful supervision appears necessary to eliminate avoidable sources of error. Various sources of error are discussed by O'Sullivan (*2*).

Cost analysis has shown that each micro blood sugar determination costs approximately 7.8 cents per test. Included in this figure are all AutoAnalyzer reagents and supplies as well as the services of one technician paid $3 per hour. This assumes that 300 determinations are made per day at a rate of 60 per hour. Depreciation, overhead, and other cost factors not included in this figure would vary with each laboratory as well as with the number of determinations made.

Previous cost studies for macro glucose determinations using the AutoAnalyzer have revealed a figure of 19 cents per test (*4*). This figure was based on 40 determinations per hour and would be reduced if a higher sampling rate was used.

In general, the micro method would be slightly less expensive than the macro method because of the use of less reagent.

The Unopette is presently available at a cost of $122.50 per 1,000. In our experience the use of the Unopette for the collection of capillary blood is less expensive and more convenient than the collection of venous blood. Technicians with less training may be employed and the entire Unopette unit is disposed of after use.

Summary

An adaptation of standard AutoAnalyzer methodology for micro blood glucose determinations using recent equipment modifications and a sample of 20 microliters of capillary blood was tested. A sampling rate of 60 determinations per hour was used. The blood sample was collected with the Unopette, a recently de-

veloped disposable capillary pipette, delivering 20 microliters of blood into a collecting reservoir containing 1.3 ml. of a 1 percent sodium fluoride solution. Studies determined that the technique demonstrated sufficient rapidity, specificity, simplicity, and economy to recommend its use as a tool for diabetes screening of large population groups. Cost analysis showed that each micro blood sugar determination cost approximately 7.8 cents per test.

REFERENCES

(*1*) Method file N-2a, N-9. Technicon Instruments Corporation, Chauncey, N.Y.

(*2*) O'Sullivan, J. B., and Kantor, N.: Variability of blood sugar levels with an automated method. Public Health Rep 78: 1023–1029, December 1963.

(*3*) Crofford, B., and Lacy, W. W.: Rapid micromethod for determination of blood sugar in mice. J Clin Med 61: 708–712 (1963).

(*4*) Greenwald, I.: Diabetes case-finding in New York City. Public Health News, New Jersey Department of Health, 126–127, April 1961.

EQUIPMENT REFERENCES

(*A*) AutoAnalyzer, Technicon Instruments Corporation, Chauncey, N.Y.

(*B*) Unopette, disposable blood diluting pipette, Becton Dickinson & Co., Rutherford, N.J.

Increase in Voluntary Retirement

More older men in good health are choosing to retire because of a preference for leisure. This is the reason given by one out of five recent retirees who were 65 or older, according to a Social Security Administration report. The report, one of a series in the 1963 Survey of the Aged, is based on personal interviews with a cross-section of men aged 62 and over, including both social security beneficiaries and those who are not. In a 1951 survey of beneficiaries, only 3 in 100 men 65 or over retired for this reason.

Comparison with the 1951 survey also shows a trend toward better health among the recently retired. Only 41 percent of the respondents gave poor health, still the most common cause, as the reason for retirement in 1963 compared with 48 percent in the earlier survey.

Of the aged men who were still working, but who planned to stop or to work less, 96 percent would be eligible for retirement benefits. This would indicate that these benefits are an important factor in voluntary retirement.

The study also shows that men in well-paid occupations are less apt to retire than those with low pay. Professional and technical workers, managers, and proprietors of businesses generally have more savings and other sources of income. However, such factors as less physically demanding work, more interesting and rewarding jobs, and better health seem to have greater influence on the decision to continue working, as does the fact that they are less likely to be subject to compulsory retirement provisions.