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Portable Simultaneous Multiple Analyte Whole-Blood Analyzer for Point-of-Care Testing

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We describe a portable clinical chemistry analyzer for point-of-care measurements of multiple analytes in <10 min from ~40 μL of whole blood (fingerstick or venous). Whole blood is applied directly to a 7.9-cm-diameter, single-use plastic rotor containing liquid diluent and ≥4–12 tests in the form of 1- to 2-mm-diameter dry reagent beads. The reagent/rotor is immediately placed in a portable instrument along with a ticket/label results card. As the instrument spins the rotor, capillary and rotational forces process the blood into diluted plasma, distribute the patient's diluted sample to cuvettes containing the reagent beads, and mix the diluted sample with the reagents. The instrument monitors the chemical reactions optically at nine wavelengths; sample volume and temperature are also measured optically. The calibration data for each reagent are read from a bar code on the periphery of each rotor. The instrument processes all the measurements to calculate, store, print, and communicate the results. Each reagent/rotor contains an enzymatic control that must be within a defined range before the results from that analysis are reported.

Additional Keyphrases: *centrifugal analyzer · glucose · urea*

During the 1960s and '70s, blood chemistry panel determination revolutionized clinical laboratory practice. Profiling began ~1963, with the SMA 6/60 and 12/60 analyzers [Technicon Instruments Corp. (now Miles Diagnostics), Tarrytown, NY 10591], followed in the 1970s by the Technicon SMAC. Concurrent with the introduction of these profiling instruments, higher-throughput discrete analyzers such as the *aca* (DuPont Medical Products, Wilmington, DE 19898) were also introduced. These instruments required well-equipped laboratories with highly trained staff, which were removed from the immediate site of patient care. Although nearly all of the large automated analyzers had "stat" features, the requirement for sample transport to the laboratory separated the procedures of sample collection and measurement in time. High-speed automated analysis was possible, but the need for rapid acquisition of essential patients' data was not being met.

To meet this need, systems for point-of-care and home use, e.g., systems for glucose, occult blood, and pregnancy testing, and certain discrete analyzers with extended menus gained rapid acceptance during the 1980s. For more comprehensive testing, we present a robust, low-cost, highly accurate blood-panel testing device that requires minimal operator skill. These characteristics make the device suitable for presenting stat results under a wide range of conditions of site or operator limitations.

Development of technology. Most blood tests require separation of interfering cellular components. Typically, separation steps are performed external to an analysis by centrifugation, followed by manual or automated pipetting of plasma or serum into separate sample cups or assay cuvettes. Because these procedures are typically labor intensive and time consuming, more-efficient automated methods have been developed.

Some prominent methods use centrifugal force to separate the blood cells from the plasma and then distribute plasma into test wells. Original work in this area was done at the Oak Ridge National Laboratory (Oak Ridge, TN), in conjunction with the National Aeronautics and Space Administration (*1–4*). The goal was to develop a clinical analyzer that would both process whole blood and perform multiple chemical analyses for use in a space station. Current embodiments of these concepts by Abbott (Vision™; Abbott Labs., North Chicago, IL 60064-4000) and as proposed by Eastman Kodak (Rochester, NY) (*5*) perform only a single test per sample application.

Other commercially available technologies designed to achieve both whole-blood processing and analysis in a single device use filtration and (or) capillary forces to separate the cellular components from the plasma for analysis. These include devices developed by Biotrack (Mountain View, CA) (*5, 6*), LifeScan (Milpitas, CA), and ChemTrak (Sunnyvale, CA).

Design goals. The system that we devised for a simultaneous multiple-analyte, whole-blood analyzer for point-of-care testing includes the following features:

- The expertise required of the operator is limited to obtaining the sample from the patient and applying an unmeasured volume (40–100 μL) of whole blood to the sample port of the rotor.
- The system performs all the sample processing and chemical analysis.
- A panel of 12 to 20 tests is performed on the same small sample of blood.
- Once applied, the sample is completely contained, permitting safe disposal of the sample.
- The measurement system is reliable; no instrument calibration by the operator is ever required.
- Internal quality control is automatically performed with every specimen to increase the confidence in the reported results.
- Either fingerstick or venous blood can be used. The entire processing and analytical procedure can take <10 min after obtaining blood from the patient.

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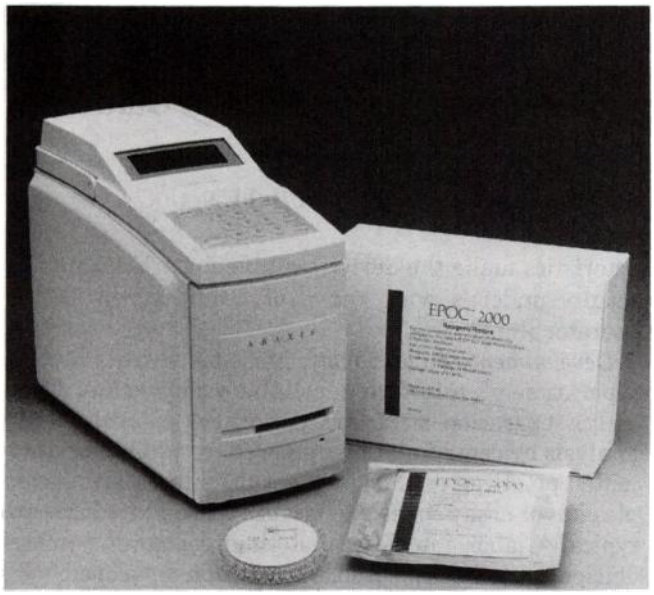

Fig. 1. Abaxis EPOC 2000 instrument and rotor containing diluent and dry reagents

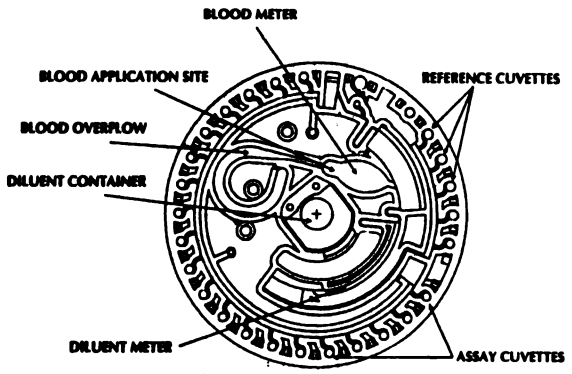

Fig. 2. EPOC 2000 plastic disposable rotor with features indicated

- The system has extended dynamic ranges to eliminate the need for repeat assays.
- The system is small and portable, allowing it to be placed in a wide variety of patient care settings.
- Regular maintenance is not needed.

Achieving these design goals required technical advances in sample processing, instrumentation, and reagents. The resulting system, the Abaxis EPOC™ 2000 (Abaxis, Sunnyvale, CA), has three parts: a plastic disposable rotor for processing the specimen, an instrument, and dried reagents deposited in the rotor (Figure 1).

Materials and Methods

Rotor

Specimen (blood) processing/analysis chambers. In the EPOC 2000, disposable, low-cost rotors process the whole-blood specimen and provide a diluted plasma for analysis. The rotor is made of molded polymethylmethacrylate plastic, 7.9 cm diameter × 2 cm thick (Figure 2); it consists of a series of many interlinked internal chambers and passages. The movement of fluid is controlled by a series of stop junctions, capillaries, and siphons managed through the use of centrifugal force. Besides the processing features, the rotor contains a diluent container set in the center of the rotor and dried reagent beads in cuvettes at the periphery of the rotor. The diluent container holds 580 μL of osmotic solution (140 mmol/L myo-inositol) to prevent cell lysis. Fluid loss from the container when in the rotor was 5 μL when stored at 8 °C for >1 year.

Cuvettes of several depths (equivalent to various optical path lengths) accommodate the various sensitivities of the assays and physiological ranges of analytes. During welding of the rotor top to the base, the consistencies of path lengths among rotors are controlled to within 5 μm (SD; n = 10 rotors with 25 cuvettes measured per rotor) to provide optical path lengths of 1.7, 2.1, 4.3, and 5 mm. Fifteen cuvettes are reserved to analyze the patient's sample; 10 are used to determine the sample dilution and internal quality-control functions.

Additionally, the rotor port for loading and metering the specimen is coated with heparin, and the outside edge of the rotor is imprinted with a bar code that contains the calibration factors. Once a specimen is applied, it remains completely contained in the rotor, permitting safe disposal.

Instrumentation

Rotor loading and positioning. The rotor containing the patient's specimen is placed in the instrument. The operator does not have to orient the rotor because, when the door is closed, the spindle captures the rotor and simultaneously opens the diluent container in the rotor. The spindle motor is closed-loop controlled to provide the specific acceleration and velocity profiles needed to achieve all of the sample-handling functions of the rotor.

Optical and signal processing. The optical unit (Figure 3) consists of a xenon arc stroboscopic lamp, the rotor's cuvettes, and a multiple-wavelength detector. The flash of the lamp is synchronized with the spinning rotor by using a cuvette position detector that senses marks molded into the rotor. About 5000 flashes are made during each analysis. The processor selects which cuvette to flash and which four of the possible nine wavelengths to measure. The multiple flashes of a cuvette are made to reduce noise through averaging.

The detector assembly contains a lens and apertures to define a viewing cone that blocks out stray light. The light is then split by a series of beam splitters and directed through the filters into the detectors. Four wavelengths are read and digitized for each flash. One of these is always 850 nm. Given the negligible absorbance from sample, reagent, or diluent at 850 nm, this signal is used to normalize the flash-to-flash variability in the strobe output. The other three wavelengths are chosen from among 340, 380, 405, 467, 500, 550, 600, or 760 nm. Use of multiple wavelengths increases the dynamic ranges and helps detect various error conditions such as nonspecific rates, abnormal reagent absorbances, and interferences.

The signal processing uses a variable-gain amplifier to compensate for lamp aging, and a 16-bit analog-to-digital converter. Full-scale values are measured for each wavelength during every analysis, so the processor

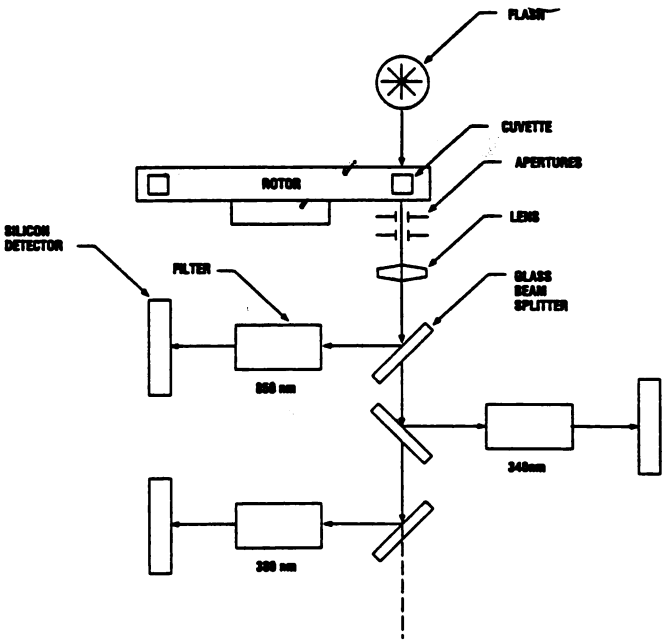

Fig. 3. Optical unit, showing light path

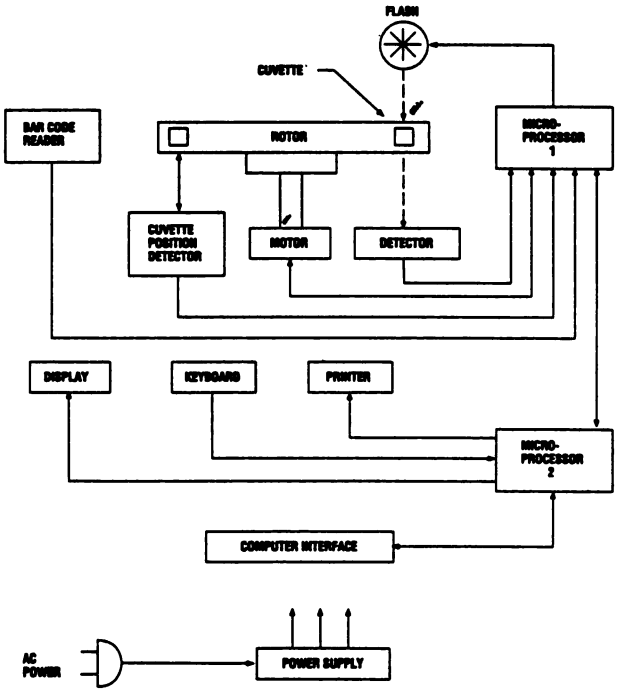

Fig. 4. Schematic function control by the microprocessors

can set the channel gain for optimum dynamic range and also check for fault conditions. The presence of a black ball in one cuvette of the rotor allows the measurement of a "dark" signal, which is used to compensate for any small offsets in the electronics and to check for fault conditions during each analysis.

Processor control and software. Two Intel 80C196 microprocessors control the functions of the instrument (Figure 4): one controls the measurement functions, the other specializes in user-interface functions. The measurement microprocessor controls the motor speed and acceleration; monitors the cuvette position detector; controls the flash lamp, the measurement electronics, and the analog-to-digital converter; and processes the raw measurement data. It also reads a bar code that is printed around the perimeter of the rotor. The bar code identifies the type of rotor and carries 70 digits of calibration information specific to the chemical lots of the reagents used in that rotor.

The interface microprocessor controls the keyboard, liquid-crystal diode display, printer functions, the loading mechanism that mechanically moves the rotor into position, and the RS-232 computer interface. The results are printed on a crack-and-peel card. Results from the last 100 rotors can be uploaded to a host computer for record keeping and billing, as well as for tracking quality-control parameters. The interface processor also completes the calculation of analytical results from the data that were transferred from the measurement processor. A user-replaceable memory card allows for easy software upgrades when rotors with new panels of tests become available.

The software allows easy use of the system with minimal training. It prompts the user to insert a rotor with an applied sample and to enter an operator number and the patient's identification number to begin an analysis. Users also have access to a menu to enter expected ranges, to choose between SI and "common" units, and to set an internal clock. Extensive error checking is done during normal operation to ensure that the instrument and rotor are functioning properly and that the data collected make sense.

Reagents

Standard method principles were used in formulating the reagents (Table 1). Attempts were made to use currently accepted method principles as well as state-of-the-

Table 1. Principles of EPOC 2000 Analyzer Methods

Analyte	Principle	Type of assay
Alkaline phosphatase	pNPP, Mg, Zn, and pH from IFCC, modified with a buffer change	Zero-order rate
Amylase	Silyl blocked G-7 substrate	Zero-order rate
Aspartate aminotransferase	IFCC (modified) with P5P	Zero-order rate
Bilirubin, total	Bilirubin oxidase	Endpoint
Cholesterol	CO/CE/Trinder	First-order rate
C-reactive protein	Immunoassay/ turbidimetry	Endpoint
Creatinine	Creatinase/Trinder	Endpoint
Glucose	Hexokinase	Endpoint
Potassium	Colorimetric	Endpoint
Total protein	Biuret	Endpoint
Urea nitrogen	Urease/GLDH/ NADH	First-order rate
Uric acid	Uricase/Trinder	Endpoint
Hematocrit	Indirect	

pNPP, *p*-nitrophenyl phosphate; P5P, pyridoxal 5'-phosphate; CO, cholesterol oxidase; CE, cholesterol esterase; GLDH, glutamate dehydrogenase; IFCC, International Federation of Clinical Chemistry.

art methods, e.g., enzymatic procedures for bilirubin and creatinine. All endpoint reactions use a sample blank: glucose, uric acid, total protein, C-reactive protein, and potassium use a common sample blank; total bilirubin and creatinine each have specific dedicated sample blanks. The reagent blanks are estimated by measuring at two or three wavelengths during the calculation of results.

For use with the rotors, we had to develop a new technology for making dried reagents. Initially, we considered such technologies as dry blending and tableting, spray drying, and fluid bed processes; however, each of these was rejected because it was difficult to achieve homogeneity of blended reagents to meet requirements of dosage precision. In our technology, the reagents are formulated as liquids, dispensed into discrete portions, and then frozen and dried with conventional lyophilization. The imprecision of dispensing for a typical lot of ~500 000 reagent portions is 1%. Each reagent is dispensed in 2.7 μL at a rate of 7800/h. The dried reagents are designed to dissolve instantly (within 5 s) and mix readily (within 2 min) with the diluted plasma. The analytical reactions begin almost concurrently with reagent dissolution by the diluted plasma.

Operational Sequence

The EPOC 2000 is simple to use. When the instrument is turned on, it automatically verifies that it is functional and is within the specified operation range. The operator applies between 40 and 100 μL of whole blood to the sample port (Figure 5*A*), using any convenient means, and verifies that sufficient sample has been applied by checking that the sample window is filled. There is no need to measure the exact amount of sample applied. The operator then puts the rotor into the instrument and enters the operator and patient identification numbers to begin the analysis.

The system performs all the sample processing and analytical steps. The instrument opens the diluent container in the center of the rotor. Once rotation starts, the bar code calibration factors are read and centrifugal force impels the diluent outward, filling an adjacent chamber (Figure 5*B*) that contains a ball coated with dye. Simultaneously, the 40 μL of blood moves into the separation chamber. Through a series of velocity changes, the dye is dissolved from the ball and mixed to homogeneity with the diluent. The rotation is stopped and a siphon fills. Upon rotation, the diluent is split into two portions, one metered (420 μL) for later dilution with the whole blood, and the other flowing to a group of cuvettes that are used to provide reference absorbances (Figure 5*C*). The rotation is stopped and another siphon fills. When rotation is resumed, the diluent transfers and mixes with the whole blood (Figure 5*D*). After the blood and diluent mix to homogeneity, the cells are separated from the diluted plasma by centrifugal forces of 525 × *g* for 1 min.

The rotation is stopped and a siphon is filled. When rotation resumes, the diluted plasma is distributed to the assay cuvettes, which contain the dry reagents (Figure 5*E*). The reagents dissolve and the reactions are initiated. After all the cuvettes are filled, their contents

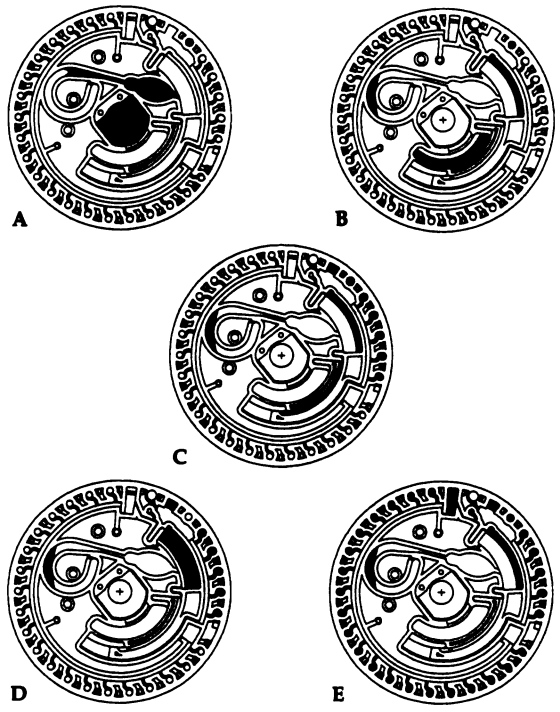

Fig. 5. Time sequence of the functions of the EPOC 2000 rotor
(*A*) Blood sample is applied; the diluent container is opened when the operator closes the instrument door. (*B*) As spinning begins, diluent enters the dye-ball mixing chamber and blood moves to the separation chamber. (*C*) The diluent is split into two portions and fills the reference cuvettes. (*D*) The measured amount of diluent enters the whole-blood separation chamber and is mixed before the cells are separated from the diluted plasma. (*E*) The assay cuvettes are filled, the reagents are dissolved, and the analysis is performed

are mixed for 2 min. The progress of the reactions is monitored photometrically.

Internal Quality Control and Calculation of Results

As far as the user is concerned, quality control within the system begins when the instrument is turned on. During an assay, the instrument continually monitors the dark current and the 100% transmittance to compensate for any optical drift.

At the completion of data collection, all the information gathered passes through a series of checks to ensure that the results are calculated from error-free information. The checks include assessment of optical and electronic drift, adequate reagent mixing, reagent integrity, substrate depletion, and temperature drift, as well as rotor functionality and proper handling since leaving the factory.

The integrity of each reagent and the quality of mixing during every run are monitored by monitoring the absorbances at several wavelengths and comparing these with expected values in the software. If values are outside the expected ranges, results are not reported for the affected methods.

One cuvette on each rotor contains a chemical method that monitors the temperature during an analysis. The temperature is monitored by a thermochromic and pH-indicating dye and is sensitive to ± 0.1 °C. The calibration of the dye is accurate to ± 0.2 °C, and its calibration factors are imprinted in the bar code on the rotor.

Each rotor also contains one cuvette for a quality-control method that is assayed simultaneously with each patient's sample. The quality-control material is D-lactate dehydrogenase (D-LDH; EC 1.1.1.28). D-LDH is not found in mammalian tissues or fluids; however, it is sensitive to the same stresses as are the coupling and analytical enzymes and labile chemicals used in the other clinical chemistry reagents. After an analytical run, the D-LDH rate is compared with an expected range entered through the bar code for that specific rotor. If the result is outside the acceptable range, no results are reported. An out-of-range D-LDH value can indicate either a problem during the run or improper handling of the rotor since leaving the factory. The D-LDH results from the last 100 runs are stored and can be retrieved or loaded to another computer for trend analysis and for preparing quality-control charts.

The sample-to-reagent ratio is measured by dilution of a dye in the diluent and is calculated from the ratio of absorbances from four of the reference cuvettes and four of the assay cuvettes. Reagent-to-sample ratios between 10 and 28 can be used.

The reaction responses are monitored for completion of endpoints, substrate depletion, effect of interferences, and nonlinearity. For endpoint methods, comparison of the absorbances from several reaction times assesses their completeness, and the absorbance value is used to monitor substrate depletion.

For rate methods, results are evaluated through our "smart kinetics" routine. A series of sequential rates are determined from segments of the reaction response. The fastest rate is determined and verified to be in the appropriate absorbance range and to have the appropriate variance. The number of observations used to calculate the rate is optimized through variance analysis, and the best rate is determined. By checking the series of rates against these criteria, potential substrate depletion, lag phases, and the role of certain interferences can be monitored and used to eliminate reporting of erroneous results. The use of multiple wavelengths and of this method of "smart kinetics" also allows us to extend the dynamic ranges of the analytes.

Because the operator never sees the patient's plasma or serum, the instrument must monitor the optical quality of each sample. Using procedures that have been modified from those proposed by Glick et al. (*8*), the instrument determines for each sample the turbidity index, the amount of icterus, and the amount of hemolysis. This information is used to estimate possible interferences for various analyses.

Results and Discussion

Only partial and preliminary performance data are currently available. As of April 1992, 14 instruments have been built; six of these are in heavy use, and each has run ~3000 rotors with no interventions. (The expected use is 5000 rotors in 3 years in normal service at a physician's office.)

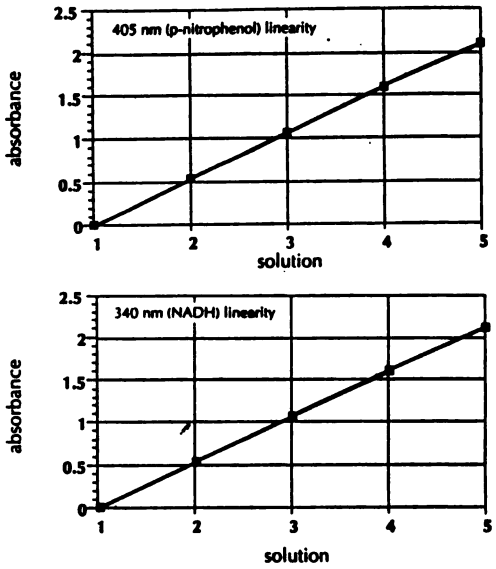

Fig. 6. Linearity of the photometer, demonstrated by measuring standard solutions of (*top*) *p*-nitrophenol and (*bottom*) NADH
Duplicate samples were measured in 4.3-mm cuvettes with three instruments

Table 2. Photometer Imprecision of EPOC 2000

Absorbance			
Mean	Total SD	CV, %	Optical path length, mm
p-Nitrophenol, 405 nm[a]			
0	0.003	—	4.3
0.534	0.0028	0.5	4.3
1.600	0.0028	0.2	4.3
2.100	0.0040	0.2	4.3
NADH, 340 nm[b]			
0.20	0.0006	0.3	1.7
0.736	0.0037	0.5	1.7
1.62	0.0022	0.1	4.3
2.140	0.0018	0.1	4.3

[a] *p*-Nitrophenol in 1-amino-2-methyl-1-propanol buffer, pH 10.3. No. of observations: three instruments with n = 6, 20 flashes per absorbance.
[b] Sample: NADH in Tris buffer. No. of observations: three instruments with n = 6, 20 flashes per absorbance.

Photometer Performance

When the absorbances of standard *p*-nitrophenol solutions are measured at 405 nm, the results of the photometer vary linearly with the concentration of the solution to >2.1 *A* (Figure 6, top). The accuracy of measuring the standard *p*-nitrophenol solutions at 1.0 *A* is ± 4 m*A*. A similar study with NADH at 340 nm also found the photometer to operate linearly to >2.1 *A* with an accuracy at 1.0 *A* of ± 5 m*A* (Figure 6, bottom).

The imprecision of the photometer at 340 and 405 nm was <4 m*A* between 0 and 2.1 *A* (Table 2). The imprecision was independent of the path lengths used.

Method Performance

We examined the dynamic ranges of several methods by using pools of human serum with added glucose and urea (Figure 7). We also examined the imprecision of several methods by assaying commercial serum controls on multiple days on several instruments (Table 3).

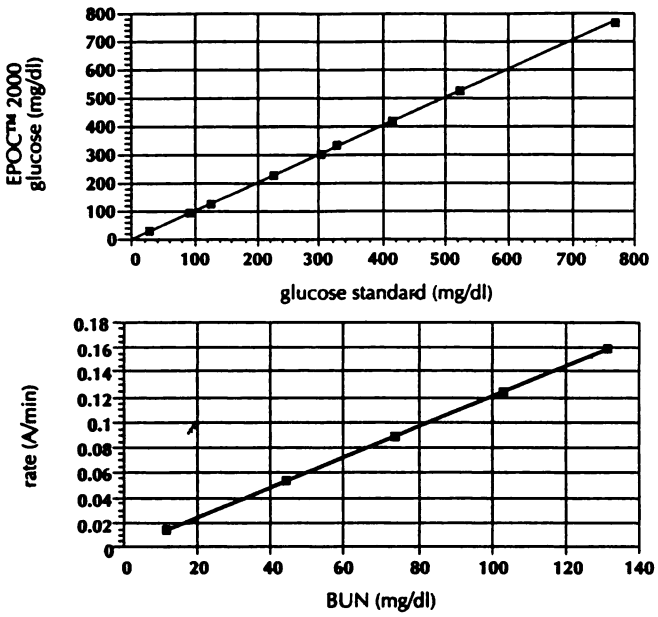

Fig. 7. Linearity of responses vs concentration for the glucose method, monitored at 340 and 380 nm in a 1.7-mm cuvette (*top*), and for blood urea nitrogen, assayed in a 2.1-mm cuvette (*bottom*)

Glucose in human serum was assayed in triplicate at a sample-to-reagent ratio of 1 to 18. Five equally spaced concentrations of urea nitrogen in human serum were assayed in duplicate on 2 days, also at a sample-to-reagent ratio of 1 to 18

Table 3. Imprecision of Two Assays on EPOC 2000

	Concn, mg/L	
Mean	SD	CV, %
Glucose (n = 20)[a]		
1010	30	3.0
2400	169	7.0
Blood urea N (n = 30)[b]		
170	10	5.9
470	24	5.2

Sample: Ciba Corning Multiqual Controls.
[a] Two instruments. 1 g/L = 5.551 mmol/L.
[b] Three instruments. 1 mg/L urea N = 0.0357 mmol/L urea.

Glucose. The glucose method yields a linear standard curve for concentrations as great as 44.4 mmol/L (8.00 g/L) when a combination of absorbances at 340 and 380 nm are used. The sample-to-reagent ratio used was 1 to 18. The glucose results are calibrated from the molar absorptivities of NADH at 340 and 380 nm. Glucose is measured in a 1.7-mm optical path and its sample blank is measured in a 4.3-mm optical path. The imprecision (CV) of estimating normal and abnormal values of glucose was 3% and 7%, respectively. To compare glucose results obtained by a Cobas Fara centrifugal analyzer (Roche Diagnostic Systems, Inc., Montclair, NJ 07042) and those by the EPOC 2000, we used heparinized plasma samples on the Cobas Fara and both heparinized plasma and whole blood from venipuncture on the EPOC 2000. The results agreed reasonably well (for whole blood $y = 1.03x + 29$, and for plasma $y = 0.9x + 50$) (Figure 8). The samples were run in duplicate, and the paired sample CV on the EPOC 2000

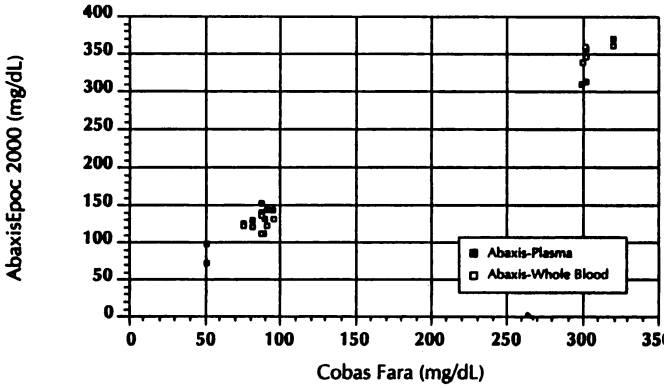

Fig. 8. Correlation of glucose values between the Cobas Fara and the EPOC 2000

Fresh human blood was analyzed in duplicate by the EPOC 2000 and the plasma obtained from the human blood was analyzed by both the Fara and the EPOC

was 1.0% for the plasma samples and 2.1% for the whole-blood samples.

Blood urea nitrogen. In part because of our kinetics approach, and in part because of our approach to reagent optimization, the standard curve for urea N is linear to 46.4 mmol/L (1.30 g/L) (Figure 7, bottom). Most commercial urea N methods are linear to ~35.7 mmol/L (1.00 g/L). The reaction response in this assay is monitored at 340, 380, and 405 nm and the cuvette for the assay provides a 2.1-mm optical path. The imprecision of estimating normal and abnormal values of urea N is 5–6% (Table 3).

In conclusion, this portable clinical chemistry analyzer can be used at the point-of-care to perform multiple analyte measurements in <10 min from ~40 μL of whole blood. The system advances the practice of clinical chemistry in the areas of instrumentation, specimen processing, reagent manufacture, and implementation of continuous quality control. The panel of tests includes endpoint methods, zero- and first-order rate methods, an electrolyte, and an immunoassay. Our goal in completing the development and implementation of the EPOC 2000 is to achieve performance comparable with that of typical laboratory analyzers.

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