



Centrifugation and capillarity integrated into a multiple analyte whole blood analyzer

In this whitepaper, the Piccolo Xpress[™] unique centrifugation and capillarity functions are described. The Piccolo processes 100 μ l of whole blood into multiple aliquots of diluted plasma and reports the results of up to 14 tests on a single reagent disc in about 12 minutes. To perform a panel of tests, the operator applies the unmetered sample directly into a single use, 8 cm diameter plastic disc which contains the required liquid diluent and lyophilized reagents. Using centrifugal and capillary forces, the disc meters the required amount of blood, separates the red cells, meters the plasma, meters the diluent, mixes the fluids, distributes the fluid to the reaction cuvettes and mixes the reagents and the diluted plasma in the cuvettes. The instrument monitors the reactions simultaneously using nine wave-lengths, calculates the results from the absorbance data, and reports the results on a convenient sticky-backed thermal roll-tape printer.

Introduction

Rapid availability of in vitro diagnostic results is advantageous to both the physician and the patient. An illness can be diagnosed more quickly and costs due to the illness can be decreased. Turnaround times are shortened, which reduces the likelihood that test results are no longer valid by the time they are received by the physician. Errors inherent in the laboratory testing cycle are also minimized by performing tests near the patient. Eliminating the need to transport samples to a central laboratory reduces such problems as misplaced samples, inaccurate labeling and transcription, improper icing and bagging, and sample degradation.

Test system must produce reliable results, independent of the user's skill, as well as shorten turnaround time and eliminate several sample handling steps. An important criterion is ease of use, since it has been demonstrated that medical office personnel can produce reliable results when using systems with simple protocols. Test systems using whole blood

and requiring no pipetting or diluting steps are also more accurate than systems which require specimen manipulation steps. This paper reports on near-patient, portable, clinical chemistry system that provides the clinician with rapid availability of in vitro diagnostic results. The Piccolo system reports results for panels of blood tests within 14 minutes of sample application to the consumable. The system consists of the single-use reagent disc and the Piccolo analyzer.

An operator applies a few drops of capillary or venous whole blood to the reagent disc and places it into the analyzer. Using capillary action and centrifugal force acting from the center of the disc, the system completes all sample processing and optically analyzes the cuvettes for multiple chemistries simultaneously. The panel of results is printed on an adhesive-backed card for easy application to patient charts. The design of this system, with emphasis on the consumable, which converts the unmetered blood sample into multiple aliquots of precisely diluted plasma, is described here.

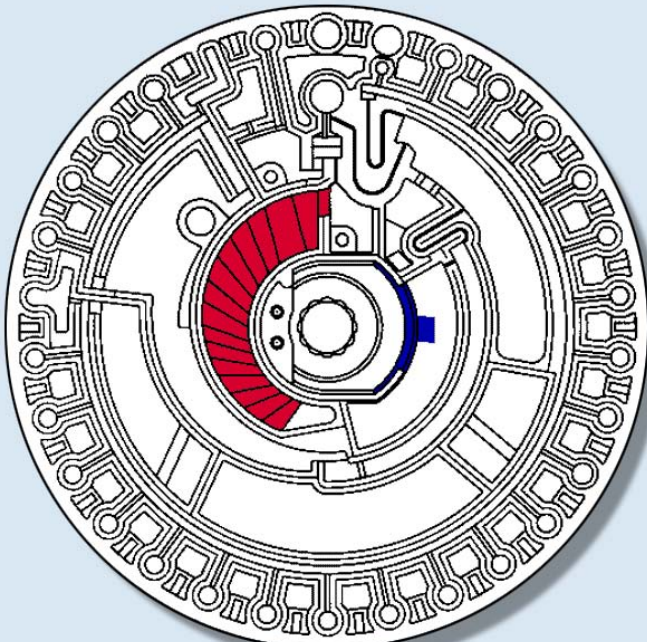
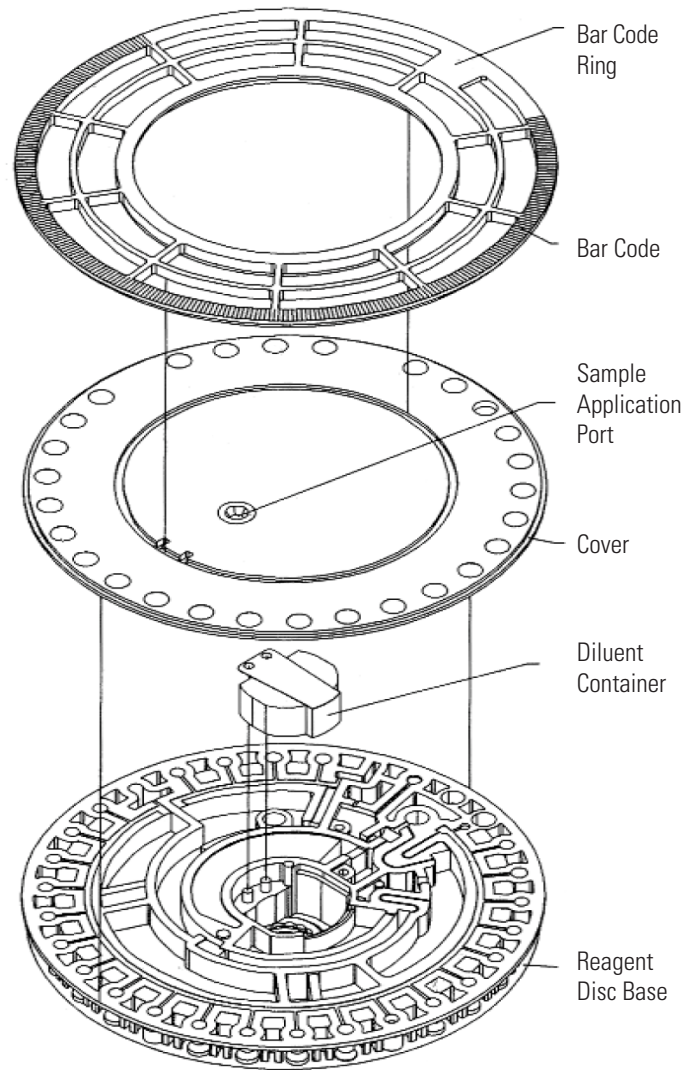


MATERIALS AND METHODS

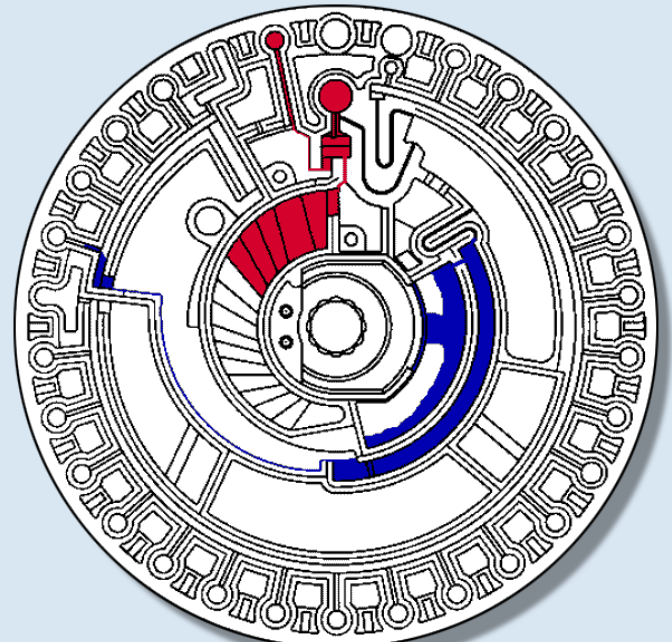
The reagent disc

The reagent disc is an 8 cm diameter consumable containing all the required diluent and dry reagents to perform a panel of tests. Three injection-molded plastic parts are ultrasonically welded together to form the reagent disc. The base and middle layer are molded from polymethylmethacrylate plastic and the top layer is molded from ABS plastic. The welded base and middle layers form the cuvettes, chambers and passageways which allow the fluids to be processed. The top layer protects the cuvette windows from fingerprints, prevents contamination of the analyzer by any sample spilled on the disc surface, and provides imprinted bar-coded, disc-specific calibration information to the analyzer.

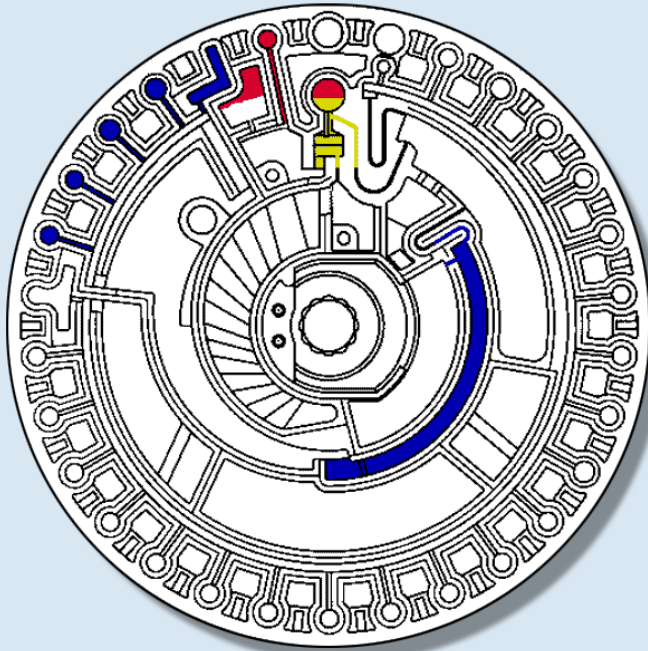
A sealed container in the center of the disc contains 474 μl of diluent. Fluid loss from the container is less than 5 μl per year when stored at 8°C. The disc contains 21 cuvettes that will be filled with diluted plasma and four cuvettes that will be filled with diluent. The cuvettes have five pathlengths (1.7 mm, 2.1 mm, 3.1 mm, 4.3 mm and 5.0 mm) to accommodate different reagent sensitivities and analyte concentrations. Pre-measured, lyophilized reagent beads for each chemistry in the panel are placed in the cuvettes at the time of manufacture.



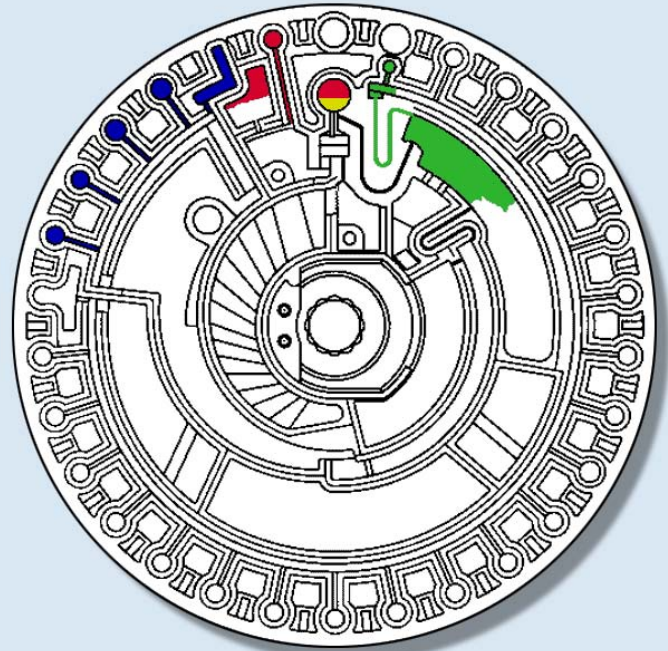
1. Schematic diagram of the disc showing the sample in the application chamber.



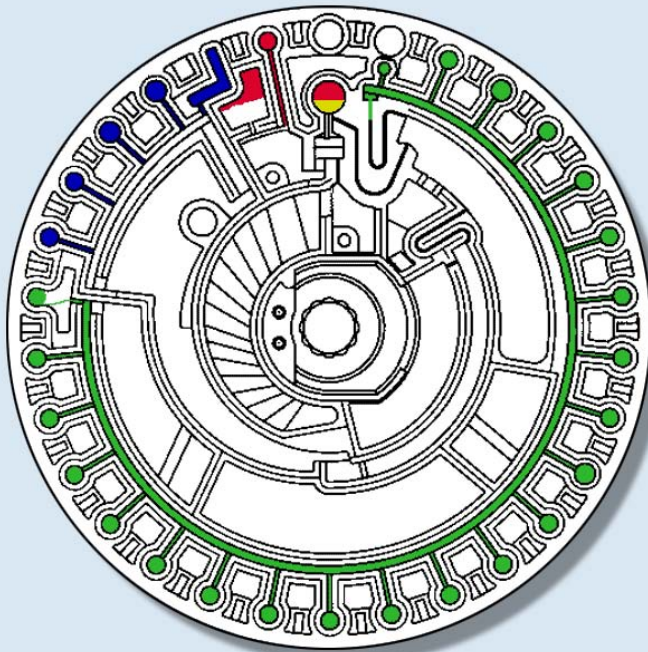
2. Schematic diagram of the disc showing blood entering the plasma metering chamber and diluent being released.



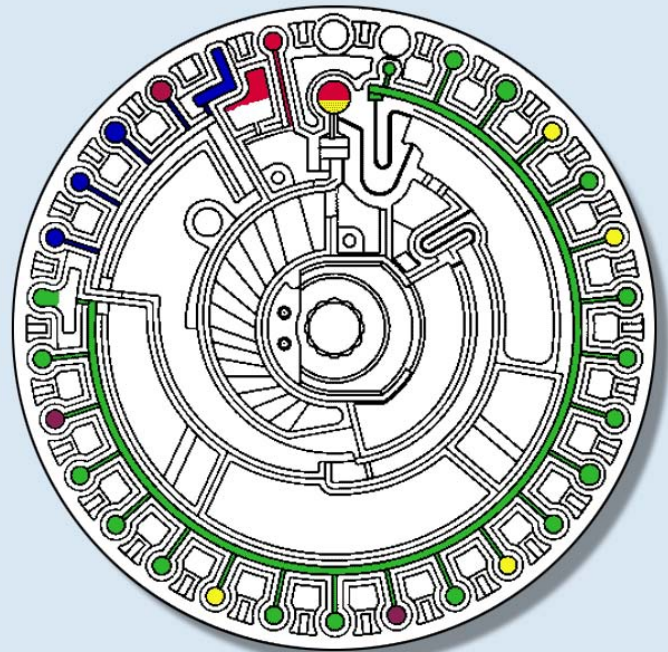
3. Schematic diagram of the disc showing the plasma separation, diluent metering, siphons primed, IOC cuvettes filled and QC checks performed.



4. Schematic diagram of the disc showing the mixing of the plasma and the diluent. Diluted plasma siphon is primed.



5. Schematic diagram of the disc showing the distribution of diluted plasma to the cuvettes containing reagents.



6. Schematic diagram of the disc showing the hydrated reagent beads and color metric IOC cuvettes.



The analyzer

The analyzer is a portable spectrophotometer measuring 32.4 cm high by 15.2 cm wide by 20.3 cm deep; it weighs 5.1 kg. A disc drawer extending from the front of the analyzer positions the disc on the center of the spindle at the beginning of each run. The analyzer's motor and controller drive the disc through the profile of rotational speeds needed to process the sample. The optical system consists of a xenon arc stroboscopic lamp and a beam-splitter/detector capable of reading nine wavelengths. A 16-bit analogue-to-digital converter is used in signal processing. Two microprocessors control the functions of the analyzer. A heater maintains the disc at $37\pm 1^{\circ}\text{C}$ during the reaction portion of the analysis.

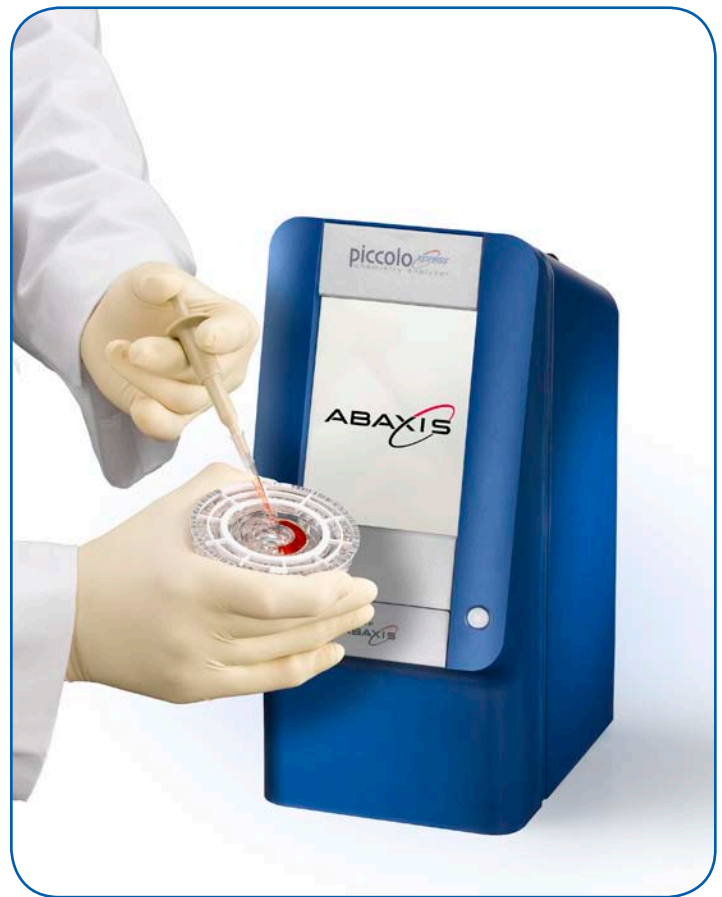
The process

To perform an analysis, the operator obtains a whole blood sample, preferably via venipuncture. Whole blood samples obtained by venipuncture can be analyzed within 60 minutes of collection. 100 μl of sample are pipetted into the disc sample port. Sufficient sample is applied to the disc when leading meniscus of the sample creates a link between two arrows printed on the disc.

The operator need not to meter the sample prior to application. A minimum of 90 μl is required and up to 120 μl may be applied to the disc. The operator places the disc with applied sample in the analyzer drawer. The drawer closes and the analyzer automatically centers the disc on the spindle. The operator inputs and places a results card in the printer. The disc is now completely controlled by the analyzer and no further involvement from the operator is required.

Transparent to the user, the diluent container is opened as the disc is loaded onto the spindle. The diluent container, a molded high-density polyethylene plastic container, is sealed with polyethylene-laminated aluminum foil. A tab on the foil is folded back across the top of the diluent container and heat staked to the disc base. A central post on the spindle enters the disc and pushes the diluent container up. This causes the foil to peel back from the edge and create an opening to release the diluent.

The sample and diluent begin the processing on separate but parallel, pathways. At the start of the analysis, the analyzer accelerates the disc to 5000



Step 1. 100 μl of whole blood is pipetted into the reagent disc.

rpm counter-clockwise and holds this speed for 2.5 minutes. Centrifugal force causes the diluent to be thrown from the diluent container into a holding chamber. The small exit channel at the most radially outward point of the chamber in a controlled manner. The chamber is filled completely with 365.25 μl of diluent. The remaining diluent overflows this chamber and moves through a channel which sequentially fills four cuvettes and places the remaining diluent into a dump isolated from the rest of the disc. The four cuvettes are used for internal disc quality control.

Simultaneously with diluent metering, centrifugal forces cause the sample to exit the application chamber and move through a small channel into the plasma metering chamber. The chamber is filled completely by 75 μl of sample and the remainder overflows into a 'sufficient sample' cuvette. Any excess sample is trapped in an isolation dump. If no fluid is detected in the 'sufficient sample' cuvette, the analysis is aborted due to a deficient quantity sample. The precise quantity of blood metered into the plasma



Step 2. Drawer is opened and full reagent disc is placed onto the receiving tray.



Step 3. After 12 minutes of processing time, the results are printed onto adhesive-backed thermal roll tape.

metering chamber is separated by centrifugal force into plasma and red blood cells. Adequate separation is achieved in 30 seconds for most samples. Samples with higher hematocrits require longer separation and packing times to provide the necessary 20 μl of clear plasma. Spinning the disc for 2.5 minutes is sufficient to separate samples up to a hematocrit of 62%.

The next step combines the diluent and the plasma. A siphon entrance of capillary dimension, which is located at the radically outermost point of the diluent metering chamber. Another siphon entrance is located partway down the plasma metering chamber. Neither of these siphons fills during the first spin because the centrifugal force is far higher than the capillary force. After the separation step is completed, the disc stops spinning and capillary forces pull the fluids over the bend in the siphons. Both siphon exit into a single mixing chamber.

The disc is now spun at 5000 rpm clockwise. The diluent metering chamber is completely emptied since

the exit of the siphon is further from the center of the disc then the extreme edge of the diluent metering chamber. The plasma metering chamber is emptied into a radial distance equaling the placement of the exit of the plasma metering siphon. The volume of plasma metered is 18.75 μl . The remaining 56.25 μl of sample and red blood cells is trapped in the lower portion of the plasma metering chamber.

The disc speed is varied to mix the metered diluent and plasma in the mixing chamber. The disc abruptly brakes to a speed of 750 rpm and then slowly climbs back to 4000 rpm, this pattern is repeated for 15 cycles. The abrupt deceleration provides sufficient tangential force to move and mix the fluid in the mixing chamber. A minimum speed of 750 rpm is required during the mixing process, to ensure that the applied centrifugal force exceeds the capillary strength of final



siphon. This mixing profile prevents priming the siphon until the fluids are homogeneously mixed.

The final siphon is primed by capillary action after mixing is complete and the disc is stopped. The disc is then spun at 3000 rpm clockwise for 40 seconds. The diluted plasma follows out of the mixing chamber and into a distribution channel which leads to 21 cuvettes and an isolation dump. The 21 cuvettes are filled sequentially and the remaining diluted plasma flows into the dump.

Each cuvette has a single channel for both flow of fluid into the cuvette and air venting. Under highly controlled conditions, the fluid flows down one side of the inlet channel while air vents from the other side. No special features of the cuvette inlet channel are required to achieve this control. The size of the fluid stream is controlled by the resistance of the siphon, the rotational speed of the disc, and the pressure head of the remaining fluid. The disc must also spin fast enough to overcome the capillary strength of the inlet channel which is 0.50 mm wide and 0.13 mm high. Each cuvette contains one for two beads of lyophilized reagents appropriate for the particular test to be run within that cuvette; these beads dissolve completely in the time required to fill the cuvette.

After all of the cuvettes are filled, and the excess diluted plasma is isolated in the dump, the disc is oscillated from 1000 rpm clockwise to 1000 rpm counterclockwise for 70 seconds. This oscillation cycle creates swirl patterns in the cuvettes which mix the chemistry and the diluted plasma.

The spectrophotometer monitors the reactions in all of the cuvettes for 3.5 minutes by flashing the xenon arc lamp synchronously with the spinning disc. The lamp is flashed approximately 5000 times for each disc.

The analyzer detects the presence of each cuvette by sensing 45 degree wedges of plastic placed every 12 degrees around the periphery of the disc. On each spin, the processor selects which cuvette to flash and which of the nine wavelengths to measure.

The results are calculated and printed on an adhesive-backed card for easy attachment to the patient's files. In addition, the results can be uploaded to a computer via an LIS/ EMR system.

THE RESULTS

The disc

The disc's contribution to total system imprecision is in the reproducibility of cuvette pathlength between discs; and the precision of metering plasma and diluent and homogeneously mixing the two fluids.

Reproducibility of cuvette pathlength

The pathlength of all 25 cuvettes was measured on 82 ultrasonically welded discs. The 82 discs include examples welded on two different welders, on different day and from different lots of molded discs. The coefficient of variation (CV) of the most precise cuvette was 0.07% and the least precise cuvette was 0.47%.

Precision of fluid metering and mixing

The goal for precision of metering plasma and diluent and mixing them homogeneously was 2% or better. The precision of the entire system was measured to obtain an indication of the precision of the disc's

piccolo xpress
Demonstration Software
MetLyte 8 Panel

25 Feb 2008 01:29 PM
Sample Type: Patient
Patient ID: 159
Disc Lot Number: 1276EXP
Serial Number: 0000P02616

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| | | | |
|------|-----|---------|--------|
| GLU | 96 | 73-118 | mg/dL |
| BUN | 15 | 7-22 | mg/dL |
| CRE | 0.9 | 0.6-1.2 | mg/dL |
| CK | 115 | 30-380 | U/L |
| NA+ | 137 | 128-145 | mmol/L |
| K+ | 4.4 | 3.6-5.1 | mmol/L |
| CL- | 103 | 98-108 | mmol/L |
| tCO2 | 26 | 18-33 | mmol/L |

QC OK
HEM 0 LIP 0 ICT 0



fluid functions, since the contribution of error from the pathlength and the analyzer was minimal. Within-day system precision was obtained using the glucose method and plasma. Four discs were run on eight analyzers for a total sample size of 32. The mean was 115 mg/dl (6.4 mmol/l) and the CV was 1.0%. Plasma was used as the sample to ensure that the glucose value would be stable for the duration of the study.

The contributions to error in this measurement include: plasma metering; diluent metering; mixing homogeneity; pathlength of reagent and sample blank cuvettes; reagent imprecision; instrument-to-instrument variation; instrument noise; and changes within the controls run on the analyzer.

The system

The reproducibility of the system for a panel of five tests was measured using Dade Monitrol ES Level I and Level II Chemistry Controls. A 15-day precision study was conducted using 12 different Piccolo analyzers. A total of 120 discs were run using each control. The day-to-day reproducibility is shown in table 1.

A 20-day precision study was conducted by the Scripps Clinic (La Jolla, CA, USA) using a single Piccolo analyzer. The study was conducted in accordance with the guidelines published by the National Committee for Clinical Laboratory Standards. The total imprecision for each method in the disc is shown in table 2.

Discussion

The precision of metering and mixing plasma and diluent is better than the 2% CV.

It has been demonstrated that a panel of clinical chemistry results can be assayed from whole blood using capillary action and centrifugal force acting from the center of the reagent disc. This is meant that a compact analyzer resulting in a portable, convenient system could be developed. The menu of tests is being expanded and several different panels will be available.





Table 1. Day-to-day reproducibility investigation tables

| Chemistry | Replicates | Mean | CV |
|---------------------------|------------|----------------------------|------|
| Glucose--normal | 120 | 70 mg/dl (3.9 mmol/l) | 1.0% |
| Glucose--abnormal | 120 | 260 mg/dl (14.4 mmol/l) | 1.6% |
| AST--normal | 120 | 40 U/l | 3.2% |
| AST--abnormal | 120 | 150 U/l | 1.7% |
| Uric acid--normal | 120 | 3.7 mg/dl (0.22 mmol/l) | 6.3% |
| Uric acid--abnormal | 120 | 7.6 mg/dl (0.46 mmol/l) | 3.1% |
| Total bilirubin--normal | 120 | 1.0 mg/dl (17.1 umol/l) | 7.5% |
| Total bilirubin--abnormal | 120 | 5.6 mg/dl (95.8 umol/l) | 5.0% |
| Total protein--normal | 120 | 6.6 g/dl (66 g/l) | 1.3% |
| Total protein--abnormal | 120 | 4.6 g/dl (46 g/l) | 2.3% |

Table 2. Total imprecision

| Chemistry | Replicates | Mean | CV |
|---------------------------|------------|----------------------------|------|
| Glucose--normal | 80 | 75 mg/dl (4.2 mmol/l) | 1.9% |
| Glucose--abnormal | 80 | 261 mg/dl (14.5 mmol/l) | 1.2% |
| AST--normal | 80 | 47 U/l | 3.9% |
| AST--abnormal | 80 | 145 U/l | 3.2% |
| Uric acid--normal | 80 | 3.8 mg/dl (0.23 mmol/l) | 4.8% |
| Uric acid--abnormal | 80 | 7.5 mg/dl (0.45 mmol/l) | 3.9% |
| Total bilirubin--normal | 80 | 1.3 mg/dl (22.2 umol/l) | 5.1% |
| Total bilirubin--abnormal | 80 | 5.5 mg/dl (94.0 umol/l) | 5.1% |
| Total protein--normal | 80 | 6.2 g/dl (62 g/l) | 2.0% |
| Total protein--abnormal | 80 | 4.4 g/dl (44 g/l) | 2.0% |

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