Patient-Side Immunoassay System with a Single-Use Cartridge for Measuring Analytes in Blood


We describe an immunoassay system suited to patient-side assay of therapeutic drugs and blood proteins. The system consists of an electronic monitor and single-use plastic cartridges containing dry reagents and liquid diluents. The monitor is turned on by insertion of a cartridge. To run the test, the user applies an unmeasured drop of blood to the cartridge when prompted by the monitor. All subsequent steps are performed without further user intervention and results are provided in less than 3 min. The system hemolyzes and precisely dilutes the blood. Hemoglobin concentration is measured; then the diluted blood is precisely diluted further and mixed with two dry reagents. The drug concentration is measured by a turbidimetric latex agglutination inhibition reaction. Theophylline and hemoglobin assay results for clinical samples correlate well with results of widely used comparison methods.

Additional Keyphrases: latex agglutination inhibition • hemoglobin • theophylline • turbidimetry

Immunoassays for analytes such as therapeutic drugs usually have complex protocols involving preparation and dilution of serum (1–5). Such assays must be done by skilled personnel in a clinical laboratory remote from the patient.

Instrumental (6, 7) and noninstrumental techniques (8) have been developed that are intended for use in the “doctor’s laboratory.” These methods still make significant technical demands on the user and require long incubation times.

Systems now available provide laboratory-quality assay results for prothrombin time (PT) (9) and activated partial thromboplastin time (APTT) within 3 min in a “user-friendly” form that does not require any action by the user once an appropriate sample has been applied.

Here we describe a method with a protocol comparable in convenience to that of the two above-named assays. It is intended to give laboratory-quality immunoassay results from a blood sample within 3 min. We demonstrate the principles of the system with a theophylline assay, using an adaptation of a commercially available chemistry.

The assay system consists of single-use (disposable) plastic cartridges containing reaction chambers, liquid diluents, and precise amounts of dry reagents, and a monitor having optics and mechanical devices that interact with the cartridge. To run the test, the user inserts a cartridge into the monitor and, after a prompt from the monitor, applies an unmeasured volume of whole blood to a well in the cartridge. All subsequent steps occur within the cartridge and are controlled by the monitor.

Materials and Methods

Description of the System

Strategy: The system is designed to perform two dilution steps in series. First, a clear hemolysate is made. In this, we measure the hemoglobin concentration of the sample, which can be used to correct the immunoassay result for hematocrit effects. The hemolysate also provides an appropriate sample for the immunoassay. A second dilution step brings the final sample concentration into a suitable range for the immunoassay (total dilution, 1:400).

Chemistry: We modified the Instrumentation Laboratory (IL) assay for theophylline in serum. This method involves two reagents that agglutinate in the absence of theophylline: a suspension of 0.1-μm-diameter latex particles to which theophylline has been linked and monoclonal antitheophylline antibody. Theophylline in the sample inhibits the agglutination and can therefore be determined turbidimetrically. To adapt this chemistry for use in whole blood, we added a diluent containing a detergent that rapidly lyses erythrocytes (see Materials). The immunoassay reagents were reformulated for application in dry form to the cartridge.

Cartridge: The reagent-cartridge is designed to dilute an unmeasured sample of blood in two distinct steps. Optical measurements of total hemoglobin concentration and of an analyte (such as theophylline) for which a suitable immunochemistry exists are made after the first and second steps, respectively. The cartridge, shown in schematic form in Figure 1, contains a set of channels and compartments that measure the required sample, remove any excess, and then dilute the sample with diluent contained in an ampule (which is broken by the monitor). Dry reagents are coated on the inner surfaces of the chambers in which optical measurements are made. Sample, reagent, and diluent are mixed by the movement of steel balls contained within the

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1 Available from Ciba-Corning Diagnostics and from Du Pont Co. as Coultrak®.
2 Available from Ciba-Corning Diagnostics.
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optical chambers. A valve is used to control movement of diluted blood between the first and second dilution steps.

The cartridge is made from three pieces of injection-molded plastic: two flat "covers" and a "body," in which most of the structure of the cartridge resides. Before the cartridge is assembled, precisely metered amounts (1–6 μL) of reagents are coated onto the "front" cover on what will become the windows of two optical cuvettes in the assembled cartridge, then dried. The "back" cover is welded to the body so that a piece of latex film is sandwiched between the body and back cover. This film serves as the movable part of the valve. Two ampules containing diluents are inserted in chambers that hold them snugly; the ampules are held securely without being crushed by the insertion of two pieces of latex, which are compressed when the cartridge is assembled. The two steel balls that will be used to cause mixing are placed into the sub-assembly. Finally, the reagent-coated front cover is welded onto the sub-assembly to form the completed cartridge.

The cartridge provides for a 432-fold dilution in two stages (1.24 × 1.18). Cartridge dimensions are: blood sample 6.4 μL; first reaction chamber 156 μL; diluted blood sample 9 μL; second reaction chamber 158 μL; optical pathlength (both chambers) 0.42 cm; mixing ball diameter 0.24 cm. External dimensions are 9 × 8.9 cm.

Monitor: The monitor has the following components: (a) light-emitting diode-optics to measure hemoglobin concentration in chamber 1 and the immunochemical reaction in chamber 2; (b) light-emitting diode-optics that detect the presence of liquids in the fluid-junction chambers and verify fluid movements through the cartridge; (c) two solenoids that break the glass ampules; (d) a solenoid that controls the valve; (e) a reciprocating motor-driven shaft, on which are mounted two permanent magnets that move directly under the reaction chambers parallel to their long axes so as to move the steel balls enclosed in the chambers to mix the chamber contents; and (f) a heating block to control the temperature in chamber 2. Data acquisition and analysis are achieved by connecting the monitor through an analog–digital converter to a computer that also controls the monitor through use of software.

System Operation

The cartridge is inserted into the monitor, where temperature control (at 25 °C for experiments reported here) and mechanical operation are performed automatically. The first step in the assay is the dilution and lysis of the blood, followed by the measurement of total hemoglobin. A sample, such as a drop (of at least 20 μL) obtained by fingerstick, is first applied to a well, which directs it into the "fluid-junction chamber" 1. Here the blood is partitioned so that it flows into a measuring capillary tube, with any surplus being drawn into a capillary drain. The dimensions of the metering capillary are precisely reproduced from cartridge to cartridge. Flow of blood stops at the distal end of the measurement capillary because of surface tension forces. After the drain has drawn all fluid from the first junction chamber, the metered sample is diluted. Diluent is released from glass ampule 1 when it is shattered by the monitor (by the moving of a solenoid). Because the diluting liquid increases the hydrostatic pressure at the end of the measuring capillary enough to overcome the surface tension, flow resumes at the end of the measurement capillary and the sample is driven into the first measurement chamber. Diluent flow continues until the measurement chamber is full. The measurement chamber is much bigger than the blood sample, so the blood is completely washed into the measurement chamber. The shape of the chamber and its surface properties are contrived such that bubble formation is precluded. The blood sample and diluent are mechanically mixed by the reciprocal movement of the steel ball within the chamber (20 s at 7 Hz). In this way a precise and accurate 24-fold dilution is achieved.

The diluent (see Materials) contains reagents that rapidly lyse erythrocytes to form a uniform solution in which the hemoglobin concentration may be determined by a measurement of absorption at 585 nm. The diluent usually contains excess potassium ferricyanide, which converts hemoglobin to methemoglobin, a suitable form for spectral measurement. The mixing chamber serves as a cuvette for the measurement, having parallel sides separated by a reproducible optical path.

Next, the hemolyzed sample is further diluted and mixed with appropriate reagents and the resulting immunochemical reaction rate is measured. During the first dilution the valve was closed by the monitor, which caused a metal solenoid to be pressed against the latex to form a seal against the cartridge body. Once the first dilution and the measurement of hemoglobin are complete, the valve is released, the diluted hemolyzed blood flows from the first measurement chamber through a capillary channel into a second fluid-junction chamber, and a sample of diluted blood is taken into a second measurement capillary. As in the first dilution, sample in excess of that needed to fill the measurement capillary is drawn into a drain until the junction chamber empties of liquid. (The valve is closed as soon as the metering capillary is full, to prevent escape of further diluted blood.) The metered, diluted sample is further diluted by displacing it from the measurement capillary with diluent 2, which is released by breaking the second diluent ampule. The sample and the second diluent fill the second reaction chamber without creating bubbles. The process essentially duplicates the first dilution except that the dilution factor is 18-fold.

After allowing the reagents in the second reaction chamber to dissolve (8 s), the diluted blood is mixed with two reagents to initiate the immunoassay. Mixing and dissolution of the reagents requires a few seconds (4 s at 7 Hz). The reaction is measured by following the increase in turbidity of the reaction mixture for 10 s.

The entire assay procedure is complete in <3 min.

Materials

Blood samples were collected into tubes containing sodium or potassium EDTA anticoagulant. The reagents for the theophylline immunoassay (IL Test, cat. no. 35229) were from Instrumentation Laboratory, Lexington, MA, but were concentrated and reformulated for use in the cartridge.

Diluent A is 0.20 mol/L glycine (sodium), pH 9.0, containing 3.0 mol of ammonium thiocyanate, 0.5 g of sodium azide, and 0.5 g of bovine serum albumin per liter. Diluent B is 2.0 g/L potassium ferricyanide solution containing 0.3 g of sodium azide and 0.5 g of Lubrol® detergent per liter. Diluent C is 50 mmol/L sodium barbital buffer, pH 8.0, containing 0.3 g of sodium azide and 0.5 g of bovine IgG per liter. The diluents were encapsulated in glass before incorporation into the cartridge. Theophylline assay cartridges contained A and B as diluents 1 and 2, respectively, and the immunoassay reagents were applied as two separate dry
patches on the part of the cartridge cover corresponding to reaction chamber 2.

Comparison Methods
Theophylline was measured with the EMIT® assay (Syva Co., Palo Alto, CA). Hemoglobin was determined by Hemocue® (Hemocue AB, Helsingborg, Sweden).

Results
Hemoglobin assay: After the dilution and mixing of blood and diluent, the erythrocytes lyse rapidly and the released hemoglobin is converted to the azide:met form. The reactions, at 25 °C, are complete within about 1 min for both diluents A and B, as judged by spectroscopic analysis (results not shown). The ability of the system to aliquot blood over a range of sample volumes was tested in the experiment described in Figure 2. Hemoglobin concentration, determined by the monitor after sample was diluted into mixing chamber 1, showed no significant change for sample volumes from 20 to 50 μL.

Blood of any hematocrit can be measured. As Figure 3 shows, the response of the system to hemoglobin concentration was linear over a range of 0–200 g/L (corresponding to hematocrits of 0–60%). Moreover, the cartridge works well with fresh blood and with blood anticoagulated with EDTA and heparin. As shown in Figure 4, hemoglobin concentration results for 20 clinical blood samples measured by this system (y) correlated well with those by a commercial technique (Hemocue, x) (y = 1.04x – 6.9, r = 0.97, SEE = 6.2 g/L; range of x: 80–180 g/L). The precision of the first dilution step can be estimated from the precision of the hemoglobin determination. At 100 and 130 g/L, the precision was 5% for 20 replicates at each concentration.

Second dilution: The system measures the immunochem-

3 Optical pathlength and monitor optics contribute to the measured imprecision. We have shown in separate experiments, where the mixing chamber is filled directly with prediluted blood, that the combined imprecision of the pathlength and optics is negligible (CV = 0.2%).

Fig. 3. Hemoglobin assay calibration
Absorbance was measured after dilution of a blood sample and recombining various volumes of plasma and erythrocytes to have a range of hemoglobin concentrations. Diluent A was used and mixing chamber 1 contained 0.24 mg of potassium ferrocyanide. The optics were referenced to the same cartridge before addition of sample.

Fig. 4. Hemoglobin assay correlation
The monitor, calibrated as in Fig. 3, was used to measure hemoglobin concentration. The comparison method (ref) was Hemocue. Triplicate values for assays were averaged.

Fig. 2. Effect of sample volume on hemoglobin assay results
The monitor was used to measure hemoglobin after applying measured volumes of blood and treating with diluent A. Determinations were made in triplicate and averaged.

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agglutination reaction rate declined by about 70%. Figure 7 shows that results by this method (\( y \)) for theophylline in 25 blood samples from randomly selected patients medicated with theophylline correlated well with those by the EMT method for the corresponding plasma samples (\( x \)). The regression equation was \( y = 1.03x - 0.25, r = 0.97, \text{SEE} = 1.2 \text{ mg/L} \).\(^4\)

Discussion

Theophylline is known to distribute predictably between erythrocytes and plasma (10, 11), so it is of little conse-

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\(^4\) Note that the assay was calibrated so as to give the plasma equivalent concentration; therefore, the slope of the regression line is expected to be unity.

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Fig. 5. Time course of the theophylline assay
The apparent absorbance of the reaction mixture in reaction chamber 2 was recorded as a function of time. Data were corrected by subtracting the absorbance for each assay immediately after completion of mixing. Samples were whole blood to which had been added 0 (□), 2.5 (○), 10 (△), 20 (□), or 40 (□) mg of theophylline per liter. The lines were calculated by linear regression analysis.

Fig. 6. Theophylline assay response
Assays as in Fig. 5. Calibrators were blood of 40% hematocrit

Fig. 7. Theophylline assay correlation
Triplicate results of assays performed in the monitor were averaged. The system was calibrated with a different set of samples for which plasma theophylline concentrations were known.

sequence to the clinical interpretation of a theophylline assay whether whole blood, plasma, or serum is used as the specimen. We selected the strategy of making a blood hemolysate as a rapid and simple way to deal with the need to eliminate formed elements in the sample before performing a turbidimetric assay. For other analytes, e.g., cyclosporin, blood is the preferred specimen (12), and for those the system described may be advantageous.

The performance of the assay in two stages permits various kinds of sample pretreatments that may be advantageous in other assays.

The system accepts the entire range of hematocrits encountered (10–60%). Any volume of sample up to 50 μL can be applied. This exceeds the maximum volume of a blood drop, so the cartridge is able to deal with any fingerstick sample. The system can recognize when insufficient blood has been applied and additional sample can be added without compromising assay performance. Planned cartridge modifications will reduce the minimum sample volume needed (20 μL) to about 15 μL so that the smallest blood drop can be used and will also provide an "overflow" at the sample application well so that very large sample volumes do not cause a problem.

The various erythrocyte abnormalities commonly found in clinical samples are not anticipated to cause problems in the assay but these issues have not yet been addressed. Samples of high viscosity should not cause problems, given that hematocrits as great as 60% are handled by the cartridge.

Assay kinetics and dose–response relationships for both hemoglobin and theophylline are essentially identical with those obtained in "mimic" assays, in which liquid reagents are used so as to give the same final assay composition. Our observations clearly show that the dried reagents used in the assay dissolve (or disperse) rapidly, without major impact on the reaction time courses. Estimates of analytical recovery of functional activity of the antigen–latex and anti-theophylline reagents after drying onto the cartridge surface, followed by addition of diluent and mixing, range from 90% to 100% (data not shown).
System performance in measuring both hemoglobin and theophylline is comparable with that of available clinical assays. We have also shown that the system can be used for analytes such as blood proteins.

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References