A Noninstrumented Quantitative Test System and Its Application for Determining Cholesterol Concentration in Whole Blood

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A novel noninstrumented technology has been developed for quantifying analytes of clinical interest in biological fluids. Application of this technology is exemplified by the development of a quantitative cholesterol test with performance equivalent to state-of-the-art instrumented methods. The assay chemistry combines two separate processes located in different areas of a test strip: enzymatic action on serum cholesterol to produce hydrogen peroxide (5 x 10 mm enzyme reagent pad) and quantification of the hydrogen peroxide (5 x 70 mm measurement region). Color bands are formed in the measurement area through the use of a redox-coupled indicator system. The height of the color band on the strip is directly proportional to the sample cholesterol concentration. A one-step cassette contains all components necessary to run the test and includes blood filtration and automatic sample measurement, so that unmeasured fingerstick whole-blood specimens can be analyzed by the non-technically trained user. The test is complete in <15 min, is read visually like a thermometer, and gives results that are in excellent correlation with established instrumented methods.

Self-testing is becoming an important part of health care as people are more aware and involved with the status of their own health (1, 2). Several qualitative or semi-quantitative self-tests—e.g., for glucose, pregnancy, ovulation, occult blood, and various urine components—have become established as physicians and patients realize their potential for providing improved health care. Recently, a noninstrumented quantitative test for measuring theophylline in whole blood was introduced by Syntex Medical Diagnostics (3). This test, AccuLevel™ is today the only test of its kind that quantifies an analyte without the use of an instrument. Although AccuLevel achieves quantitative results with good accuracy and precision, it is not ideal for use by non-technically trained personnel because it requires exact sample measurement and has a somewhat complex assay protocol.

In certain environments where tests are performed infrequently, or where the staff may not be sufficiently trained in maintaining an instrument, noninstrumented technology becomes important. In addition, the cost of an instrument may sometimes be a factor that reduces the availability of a needed test. For the broadest application, such technology should be able to handle whole blood from a fingerstick—ideally, a drop of unmeasured blood from which one can obtain a quantitative amount of plasma without the use of a pipette. Such a technology would likely be applicable to any biological fluid and thus have potential to be used with most analytes in the general chemistry and immunochemistry areas. In addition, a technology that can handle quantitative tests can also be applied to semi-quantitative or qualitative tests.

We decided to try to fill the need for a user-friendly, noninstrumented, quantitative self-test for measuring cholesterol. The Expert Panel on the Detection and Treatment of High Blood Cholesterol in Adults, National Cholesterol Education Program (NCEP), has established guidelines for adults older than 20 years to identify risk groups associated with various concentrations of blood cholesterol (4): <2000 mg/L (5.17 mmol/L) is a desirable cholesterol concentration, between 2000 and 2390 mg/L (6.18 mmol/L) is borderline high, and >2400 mg/L (6.21 mmol/L) is high.1

To assure the quality of cholesterol testing, the NCEP Laboratory Standardization Panel issued guidelines for accuracy and precision associated with laboratory cholesterol determinations (5). This same NCEP report recommended that all adults should know their cholesterol concentration, and there has been an effort to identify individuals with high concentrations of cholesterol and to initiate treatment. Desk-top instrumentation capable of performing rapid, on-site cholesterol determinations has become available, making cholesterol screening more accessible to the general public. Although these portable analyzers have increased the number of Americans who are aware of their cholesterol concentration, several limitations associated with user competence and system accuracy have been identified (6, 7).

We describe here a noninstrumented quantitative test system for cholesterol with the following performance: (a) an unmeasured drop of fingerstick blood is used, (b) the test is complete in <15 min, (c) read out is objective, as in a thermometer, (d) precision and accuracy are consistent with NCEP guidelines, (e) the test strips can be stored at room temperature, and (f) the strips are disposable after a single use.

Assay Principle

As shown in Figure 1, four paper strip components and a wicking reagent comprise the assay configuration. The measurement region, a strip of chromatography paper, contains substrates for horseradish peroxidase (HRP, EC 1.11.1.7) immobilized in a uniform coating. The paper strip area adjacent to the measurement region is the enzyme reagent pad, which contains cholesterol esterase (EC 3.1.1.13) and cholesterol oxidase (EC 1.1.3.6) immobilized

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1 Nonstandard abbreviations: NCEP, National Cholesterol Education Program; CAP, College of American Pathologists; HRP, horseradish peroxidase; MBTH, 3-methyl-2-benzothiazolinone hydrazone; and DMA, N,N-dimethylaniline.

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in a dry formulation. The next strip area is the sample-receiving pad, a disk of chromatography paper that holds 5 μL of plasma or serum. The area below the sample pad, the wicking strip, transports liquid from the area of the wicking reagent to the sample pad. The final component is the wicking reagent, a buffered protein solution containing HRP.

The entire assay chemistry is performed and completed during the capillary migration of the wicking reagent through the contiguous sections of the assay strip. The operation of the test strip is as follows: 5 μL of plasma or serum is deposited on the sample pad (Figure 1A); wicking is initiated by allowing the lower portion of the wicking strip to contact the wicking reagent (Figure 1B); the wicking reagent, containing HRP, moves the sample into the enzyme pad, where the cholesterol in the plasma is converted to hydrogen peroxide (Figure 1B, C); the hydrogen peroxide then moves with the wicking reagent into the measurement region where, in the presence of HRP, it reacts with the immobilized substrate to produce a visible color band (Figure 1C, D). The assay is complete when the wicking solution reaches the end of the measurement region, which takes about 10 min.

The hydrogen peroxide reacts stoichiometrically with respect to the immobilized substrate in the measurement area. Because the HRP substrate is uniformly distributed in this region, the height of the color band is a function of the amount of hydrogen peroxide generated in the enzyme reagent pad. In this way, a quantitative signal is produced that can be visually interpreted by measuring the height of the color band.

Materials and Methods

Cholesterol esterase, cholesterol oxidase, and HRP were from Boehringer Mannheim Biochemicals, Indianapolis, IN, and Toyobo Co., Ltd., Osaka, Japan. o-Dianisidine, 3-methyl-2-benzothiazolinone hydrazone (MBTH), and N,N-dimethylaniline (DMA) were purchased from Aldrich Chemical Co., Milwaukee, WI. Sodium cholate, Triton X-100, 4-chloro-1-naphthol, tetramethylbenzidine, 4-aminooantipyrine, p-hydroxybenzenesulfonate, phenol, sodium chloride, and sucrose were from Sigma Chemical Co., St. Louis, MO. Sodium phosphate monobasic was from Mallinckrodt, Inc., Paris, KY. Bovine serum albumin Fraction V and bovine gamma globulina Fraction II were purchased from Miles Laboratories, Elkhart, IN. Six concentrations of cholesterol calibrators were prepared in-house by supplementing fresh human serum (cholesterol = 1500 mg/L, 3.88 mmol/L) with a human lipoprotein concentrate to yield final concentrations near 1500 (3.88), 2000 (5.17), 2500 (6.47), 3000 (7.76), 3500 (9.05), and 4000 mg/L (10.35 mmol/L). Three concentrations of human serum reference material were purchased from the College of American Pathologists (CAP), Skokie, IL.

Measurement Region

This area of the assay strip of chromatography paper (5 × 70 mm) contains substrates to HRP immobilized in a uniform coating. We examined various unimolecular peroxidase substrates, including tetramethylbenzidine, o-dianisidine, and 4-chloro-1-naphthol, as well as coupled systems, e.g., 4-aminooantipyrine with p-hydroxybenzenesulfonate, and MBTH with DMA.

In this paper we report our results with the MBTH/DMA substrate system for HRP. Covalent attachment of the DMA required the synthetic addition of a primary amine to the DMA molecule. The aminated substrate molecule was then coupled to a carbodiimide-maleimide-activated chromatography paper in a manner similar to that described by Chapman and Ratcliffe (8). The MBTH was subsequently immobilized to the DMA-derivatized chromatography paper by simple adsorption.

The amount of HRP substrate immobilized in the measurement area determines the sensitivity of the assay. The color band height produced from a given concentration of hydrogen peroxide (or cholesterol) is inversely proportional to the density of immobilized peroxidase substrate. Higher loadings of substrate give lower migration heights. Because loading of substrate in this area is predictable, sensitivity can be adjusted to meet the requirements of the particular assay.

Enzyme Reagent Pad

This area of the assay strip chromatography paper (5 × 10 mm) contains reagents in a dry formulation, which convert plasma or serum cholesterol to hydrogen peroxide.

The paper is prepared by immersing a sheet of chromatography paper in a phosphate-buffered pH 7.0 solution containing, per liter, 18 kU of cholesterol esterase, 50 kU of cholesterol oxidase, 10 g of sodium cholate, and 5 g of sucrose. The paper is then dried.

Because the cholesterol esterase and cholesterol oxidase are not irreversibly immobilized to the chromatography paper, they move slowly upward during wicking. After wicking is complete, these enzymes are spread upward from the enzyme reagent pad 10 to 15 mm into the lower part of the measurement region. Most of these enzymes, however, remain on the enzyme reagent pad throughout the wicking process.

For the cholesterol enzymes, 1 U of activity forms 1 μmol of hydrogen peroxide per minute. For this activity assay we used 4-aminooantipyrine with phenol and Triton X-100 in 0.10 mol/L potassium phosphate buffer, pH 7.0, as described in the Toyobo catalog (sections C00-311 and COE-311).
Sample Receiving and Wicking

The sample-receiving pad is a 5-mm-diameter disk, the wicking strip is 5 × 22 mm. Both are untreated chromatography paper. The wicking reagent contains 5 mg of HRP and 2 g of bovine gamma globulin per liter of 100 mmol/L phosphate buffer (pH 7.0).

To eliminate edge effects commonly associated with paper chromatography, we cut the lengthwise edge of the measurement region with a special die that produces serrations. This serrated-edge configuration results in rocket-type color fronts; their convex-rounded leading edge makes the color band height easy to read (Figure 1). This method has been previously described by Zuk et al. (3).

AccuMeter® Cassette System

The solid-phase reagent chemistry described above has been enclosed in a cassette (Figure 2), making it easier for nontechnically trained individuals to operate the test. This cassette system, the AccuMeter (patents pending), includes a blood separation device, a sample measurement system, and a mechanism for release of wicking reagent.

Because all the necessary reagents, the blood-separation system, and the pipette are self-contained in the cassette, the only additional part needed to conduct the test is a finger lance. The test procedure is very simple: the user lances a finger, applies a drop of blood to the sample application site, and waits about 1 min before pulling the slide to the right. About 10 min later, the "test-complete" window turns green, indicating that the result can be read. The height of the color bar is read in millimeters and compared with a conversion table to obtain a quantitative result. The entire cholesterol determination is complete in <15 min.

Figure 3 shows an exploded view of the AccuMeter test system, illustrating the relative position of internal components. The cassette is composed of three plastic parts: a cover, a movable slide, and a base. The assay strips are positioned, as indicated, with the measurement region, the enzyme reagent pad, and the wicking strip located on the right side. The sample pad is positioned in the movable slide. A sealed buffer container on the underside of the cassette cover is directly above the slide ripper teeth (its position is outlined in Figure 3A).

When the blood sample is applied, the slide is positioned such that the sample pad is aligned with the blood-separation device and the sample well on the cassette cover (Figure 3A). Sample filtration proceeds, removing erythrocytes and depositing the plasma on the sample pad (this takes about 1 min). The cassette slide is then pulled to the right (Figure 3B), which accomplishes two things simultaneously: it aligns the sample pad with the assay strips, and it releases the wicking reagent by drawing the ripper teeth across the buffer container and rupturing its seal. Sample measurement is also complete in that, at this point, a reproducible volume of plasma is delivered into the assay system regardless of the volume of the whole-blood sample initially applied to the cassette.

In-House Cholesterol Comparison Assay

We use the Boehringer Mannheim High Performance assay as our in-house comparison system for cholesterol. Comparison of this assay performed in-house (y) with the Abell–Kendall assay (9) performed in a national reference laboratory (x) showed virtually identical results. The regression line equation associated with this comparison was

\[ y = 0.996x - 1.03 \]  

\( r = 1, n = 18 \).

Results and Discussion

Sample Preparation

Automatic sample measurement is a significant feature of the cassette. We examined the performance of the sample-measurement device by challenging the cassette system with blood volumes from 5 to 50 μL and then measuring the weight of the sample-recovery pad. As Figure 4 shows, 20 to 25 μL of whole blood is necessary to saturate the metering system; thereafter, plasma recovery is constant. Thus, when whole-blood sample volumes exceed 30
μL, the plasma volume obtained is 5 μL (SD ranging from 0.08 to 0.15 μL). Typically, the coefficient of variation (CV) of this sample-measurement system is 2%.

Because the sample-measurement system operates at volumes >30 μL, the blood-separation system must also remain effective under the same conditions. We evaluated the performance of the blood-separation device by challenging the system with a range of whole-blood volumes and then measuring the hemoglobin concentration of the contents of the sample-recovery pad. A properly functioning blood-separation system would show only low concentrations of hemoglobin on the sample pad.

Figure 5 compares the performance of two methods of erythrocyte separation: the AccuMeter procedure and the widely accepted method of blood separation by filtration through glass fibers. In the typical glass fiber system, 30-μL samples give acceptable performance with low concentrations of hemoglobin found on the sample pad; at greater volumes of whole-blood samples, however, hemoglobin recovery increases dramatically. For example, applying 50 μL of blood to the glass fiber system results in hemoglobin (49 g/L) accumulating on the sample pad. This is not acceptable because hemoglobin at concentrations >1.0 g/L interferes with our assay chemistry.

The effectiveness of the blood-separation device for the AccuMeter system, on the other hand, is practically independent of sample volume. This device combines glass fibers with a microporous membrane and has performance superior to previously described methods of static blood separation (10, 11). Applying 50 μL of blood yielded only 0.40 g of hemoglobin per liter on the sample pad (Figure 5).

Calibration Curve

Each lot of AccuMeter test cassettes is calibrated by using a six-concentration calibrator set for serum cholesterol. The six calibrators are run in replicates of 5 over three consecutive days, resulting in 15 replicates for each calibrator concentration. The mean of replicates at each concentration is calculated and the results are plotted as a master curve (Figure 6). By this method of calibration we established the dynamic range of the cholesterol assay to be from 30 to 4000 mg/dL (10.35 mmol/L). Accurate results will be obtained when sample concentrations are within this range.

Accurate calibration of cholesterol measurement methods has historically posed a significant challenge, largely because of method-specific matrix effects in various cholesterol calibration materials. Processed calibration materials often do not give the same results as patients' fresh samples, but are either higher or lower than the "true" (Abell-Kendall) value for a fresh sample.

Accuracy

Accuracy can be established in only one way (12), an iterative process involving: (a) direct comparison of the cholesterol assay in question to the reference Abell-Kendall method by analyzing patients' fresh samples, (b) calibration set-point adjustment as indicated by step a, and (c) a blind correlation analysis between the cholesterol assay and the reference Abell-Kendall to verify an acceptable bias. We followed this process to calibrate the AccuMeter system and thereby established accuracy through traceability to the National Reference System for Cholesterol. Because the Abell-Kendall reference assay (9, 13, 14) must be performed in one of the nine laboratories that are members of the National Reference Method Laboratory Network (12), we determined accuracy of the AccuMeter system by correlation to the in-house Boehringer Mannheim High Performance reference assay. Figure 7 shows the values obtained for EDTA-treated blood assayed with the AccuMeter plotted versus plasma values obtained with the comparison system. The regression line equation of results for 74 patients' samples shows a slope very close to 1.0 with a small negative intercept of 13.3 mg/dL (0.03 mmol/L) and a correlation coefficient of 0.97.

We also evaluated accuracy by using an instrument that accepts EDTA-treated whole blood samples. For this, we used the Abbott Vision (Abbott Laboratories, N. Chicago, IL), which has been demonstrated to give accurate results.
(6). Correlation between the two methods was excellent, as was the correlation for fingerstick whole blood (Figure 8).

Although additional clinical evaluations are needed, especially in the area of fingerstick determinations, these data clearly demonstrate the accuracy of this noninstrumented system. The lack of unacceptable bias also validates the calibration method used for AccuMeter, which is traceable to the National Reference System for Cholesterol.

**Precision**

The precision of the AccuMeter chemistry is primarily a result of the coating uniformity of the peroxidase substrate in the measurement region. The uniformity of this paper is tested by wicking the measurement region by immersing only the lower 5 mm of the 70-mm strip in 0.5 mL of a buffered protein solution containing 5 mg of HRP and with 2.6 mmol of hydrogen peroxide per liter. The precision of color band height produced is an estimate of the substrate uniformity in this strip region, independent of all other strip areas. The SD of migration height is 0.5 mm (CV = 2%).

The overall AccuMeter precision is influenced by other system components as well, including the automatic sample-measurement device and the uniformity of the enzyme reagent pad. It is difficult to estimate the real contribution to precision from each system component; however, we have determined the within-run and between-run precision for the complete AccuMeter test system.

The within-run precision was determined by assaying EDTA-treated blood in replicates of 20 at four cholesterol concentrations. As Table 1 shows, at cholesterol concentrations of clinical interest, the assay precision ranges from 4% to 5%.

The total analytical precision was estimated by running five replicates on each of nine separate days over a three-month period. Because whole blood is not stable over the time frame of this study, we used CAP reference serum, reconstituting a new bottle of lyophilized reference material with 3.0 mL of water at each time (Table 2). One way analysis of variance was used to estimate the within-run and (corrected) between-day components of the total variance (15). These components were then added together to estimate the total analytical precision.

**Temperature Effect**

The effect of ambient temperature on the assay chemistry was determined by performing assays at 4, 18, 23, and 37 °C with the CAP cholesterol calibrator (1470 mg/L, 3.8 mmol/L) in replicates of five at each temperature. There was no change in migration height or assay quantification at these temperatures.

**Table 1. Within-Run Whole-Blood Precision**

<table>
<thead>
<tr>
<th>Cholesterol concn, mg/L</th>
<th>Mean</th>
<th>SD</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1680</td>
<td>69</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>1930</td>
<td>99</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>2550</td>
<td>123</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>2650</td>
<td>115</td>
<td>4.3</td>
<td></td>
</tr>
</tbody>
</table>

*EDTA-treated blood run in replicates of 20 at four cholesterol concentrations.

**Table 2. Total Analytical Precision**

<table>
<thead>
<tr>
<th>Cholesterol concn, mg/L</th>
<th>Day</th>
<th>Mean</th>
<th>SD</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2530</td>
<td>88</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2610</td>
<td>95</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>2620</td>
<td>123</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>2760</td>
<td>98</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>2500</td>
<td>63</td>
<td>2.5</td>
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<td></td>
<td>28</td>
<td>2550</td>
<td>143</td>
<td>5.6</td>
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<td></td>
<td>42</td>
<td>2560</td>
<td>95</td>
<td>3.7</td>
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<td></td>
<td>56</td>
<td>2590</td>
<td>145</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>85</td>
<td>2610</td>
<td>100</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>2580</td>
<td>109</td>
<td>4.2</td>
</tr>
</tbody>
</table>

CAP reference serum was analyzed in replicates of 5 on nine separate days over an 85-day period. Variance components analysis was performed (15) and total analytical precision was determined as follows: grand mean of all results = 2584 mg/L. Within-run variance = 1139, SD = 106.7, CV = 4.13%. Between-day (corrected): variance = 269 - (1139/5) = 41.2, SD = 20.3, CV = 0.79%. Total analytical precision variance = 1139 + 41.2 = 1180.3, SD = 109, CV = 4.2%.
Interfering Substances

Table 3 shows the threshold concentration of interfering substances (the concentration at which there is no measurable effect on assay quantification). Concentrations greater than those listed may cause interference; however, in most cases, the concentrations listed were the highest tested. Most of the potential interfering substances were water soluble, so that we could add incremental amounts of the material to the control serum. The AccuMeter migration height for unsupplemented serum was compared with the migration height for the sera supplemented with potential interferents.

To evaluate triglyceride and bilirubin interference, we used lipemic and icteric clinical samples. The cholesterol concentrations of these samples were determined with the Boehringer Mannheim comparison assay as well as the AccuMeter. The interference associated with bilirubin is unclear because the comparison assay may be subject to interference from this compound at concentrations >40 mg/L (0.068 mmol/L). The AccuMeter test does not show interference at this concentration. Further evaluations by using the Abell–Kendall reference procedure (which is subject to little bilirubin interference) need to be done to determine the actual amount of interference from this compound. At present, we list 40 mg/L (0.068 mmol/L) as the bilirubin threshold, but experiments with further additions of bilirubin suggest that the threshold is well over 100 mg/L (0.17 mmol/L). The triglyceride threshold listed in Table 3, 3600 mg/L, is the highest concentration of lipemic sample we tested. Although this concentration is clinically insignificant, we do not expect to see interference from lipemic samples.

Additional studies are underway to evaluate potential interference effects of cholesterol-lowering drugs, common prescription drugs, over-the-counter products, and metabolites of these compounds.

Stability

Stability has been evaluated for component parts as well as the complete cassettes. All materials were sealed in a

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Table 3. Interfering Substances

<table>
<thead>
<tr>
<th>Substance</th>
<th>Reference range*</th>
<th>Threshold concn, b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>10–40 mg/L</td>
<td>1000</td>
</tr>
<tr>
<td>Ascorbate</td>
<td>6–20</td>
<td>100</td>
</tr>
<tr>
<td>Triglycerides (lipemic sample)</td>
<td>350–1600 mg/L</td>
<td>3600</td>
</tr>
<tr>
<td>Bilirubin (icteric sample)</td>
<td>1–11 mg/L</td>
<td>40</td>
</tr>
<tr>
<td>Creatinine</td>
<td>5.0–12</td>
<td>1100</td>
</tr>
<tr>
<td>Estradiol</td>
<td>5×10^-4</td>
<td>0.1</td>
</tr>
<tr>
<td>Progesterone</td>
<td>0.1</td>
<td>0.15</td>
</tr>
<tr>
<td>Iodoacetic acid</td>
<td>2000</td>
<td>5000</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>8000</td>
<td>12 000</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>1.6–3.5</td>
<td>500</td>
</tr>
<tr>
<td>Oxalic acid</td>
<td>2.4</td>
<td>7000</td>
</tr>
<tr>
<td>Sodium fluoride</td>
<td>4200</td>
<td>4200</td>
</tr>
<tr>
<td>Uric acid</td>
<td>7</td>
<td>10 000</td>
</tr>
</tbody>
</table>

* The reference range is the concentration normally found in serum. b At the threshold concentration, no change in assay performance is seen. All threshold values except hemoglobin and ascorbate were the highest concentrations tested. c These are the concentrations found in blood-collection tubes.

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Table 4. Assay Stability

<table>
<thead>
<tr>
<th>Component</th>
<th>Storage condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measurement region</td>
<td>8 months, 45 °C</td>
</tr>
<tr>
<td>Enzyme reagent pad</td>
<td>6 months, 37 °C</td>
</tr>
<tr>
<td>Wicking reagent</td>
<td>6 months, 37 °C</td>
</tr>
<tr>
<td>Complete cassette</td>
<td>1 month, 45 °C</td>
</tr>
</tbody>
</table>

* All assay components were stored in sealed foil pouches with desiccant.

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In summary, this novel noninstrumented technology can, in one step, separate plasma from whole blood, measure sample volume, and obtain accurate and precise quantitative results. Such a technology has potential application for a wide range of analytes in areas of general chemistry and immunodiagnostics. The total cholesterol assay described here achieved performance substantially equivalent to state-of-the-art instrumented methods, with excellent stability and a convenient protocol, making it ideally suited for on-site consumer use in the physician’s office and in the home. It should contribute to identifying persons at risk for coronary artery disease by being available to and usable by a broad spectrum of users.

The exceptional performance of this noninstrumented assay system is possible through helpful discussions and meticulous attention to fine detail from: Sheng Li, Jerry Gin, Pyare Khanna, Badri Dasu, Sunita Badam, David Wibbelsman, Charles Cypher, Lan-Thanh Huynh, Helen Chaw, D. Elaine Belardi, Joanne Miller, John Lankford, Paul Patel, Doug Rundle, Don Blanding, Maura O’Brien, Chris Kitazawa, and Edward Sassi. We gratefully acknowledge their assistance.

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