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Integration of Paper-based Microfluidic Devices with Commercial Electrochemical Readers

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ABSTRACT

The combination of simple Electrochemical Micro-Paper-based Analytical Devices (EµPADs) with commercially available glucometers allows rapid, quantitative electrochemical analysis of a number of compounds relevant to human health (e.g., glucose, cholesterol, lactate, and alcohol) in blood or urine.

Keywords: Glucometers, microfluidics, electrochemical sensing, medical diagnostics
INTRODUCTION

This article describes a simple, electroanalytical system — based on the combination of a commercial hand-held glucometer with easily fabricated Micro-Paper-based Analytical Devices (μPADs) — that is useful in quantitative analysis of metabolites such as glucose, cholesterol, and lactate in human plasma or whole blood, and ethanol (or acetaldehyde) in aqueous solution. These electrochemical devices (which we call Electrochemical μPADs or EμPADs) provide fluid handling, and support sensing electrodes; an inexpensive, commercial electrochemical reader (a glucometer) carries out electrochemical analyses and displays the results in digital format. The glucometer is an amperometer that is used to measure the quantity of an electroactive species formed by the reaction of glucose with reagents stored in the test strips. The EμPADs include microfluidic channels, electrodes, and electrical interconnects fabricated in chromatography paper using wax printing and screen printing (Figure 1A, B). The wires were printed using silver ink, and four electrodes (a working electrode, a counter electrode, and two internal reference electrodes) were printed using graphite ink.

The chemical reagents needed for the assays of glucose and alcohol were stored in the detection zone of the EμPAD. To use this system, we usually inserted the dry EμPAD into the port of the glucometer. After applying a drop of fluid to the exposed end of the EμPAD, and allowing liquid containing the analytes to wick to its sensing region, the glucometer initiated amperometric measurement, and displayed the electrochemical readout on its LCD screen (Figure 1C). In some reactions (e.g., those for lactate and cholesterol), when the time interval required to complete the enzymatic reactions was greater than the 10-second waiting-time set in the glucometer, we mixed the solution of
**Figure 1.** Components of an E\(\mu\)PAD-based system that uses a commercial glucometer as an electrochemical reader. A) Arrays of microfluidic paper channels fabricated in chromatography paper using wax printing, and an enlarged image of one paper channel (left), and a representative microfluidic paper device with electrodes and electronic wires fabricated using screen printing (right). The number of devices that could be fabricated on one US letter-sized page was approximately 150 - 200. B) A photograph of a commercial test strip made from plastic (left), and an E\(\mu\)PAD made from a single layer of paper (right); chemical reagents were stored in dry form in the detection zone in the dashed square. C) The glucometer used as a reader. An E\(\mu\)PAD was inserted into the test port with the contacts and the display facing up. After applying an aqueous solution containing analytes, the glucometer displayed the electrochemical readout on its LCD screen.
Figure 1

A) 

B) 

C) 

stored reagents

silver circuits

wax

carbon electrodes

2 cm

5 mm

CVS/pharmacy

TRUEtrack™

05/17 6:43 PM

119 mg/dL
analytes with the chemical reagents needed for the assays in a small centrifuge tube (the mixing can also be conducted on any clean substrate, such as a plastic thin film, or the surface of a table), and allowed the reaction to proceed to completion. We then inserted a dry EµPAD into the port of the glucometer, and dipped the exposed end of the EµPAD into this reacted solution to perform the analysis.

This work is part of a broad effort to develop high-quality, low-cost biomedical analyses appropriate for use in the developing world, and in resource–limited settings. Here we take advantage of a highly developed and commercially successful technology—the electrochemical quantification of glucose in blood—for other applications. We believe that these electrochemical systems possess the characteristics required to be useful in a range of applications, including human, animal and plant diagnostics, food-quality control, and environmental monitoring.

The technology used in the currently available glucometer is based on the quantitation of electron-transfer mediators (e.g., ferrocyanide) generated by the enzyme-catalyzed oxidation of glucose (eq. 1, GOx is glucose oxidase)

\[
D\text{-glucose} + H_2O \xrightarrow{\text{GOx}} \text{Gluconic acid} + 2H^+ + 2e^-
\]

\[
2\text{Fe(III)}\text{CN}_6^{3-} \rightarrow 2\text{Fe(II)}\text{CN}_6^{4-}
\]

Our objective here is to demonstrate that glucometers have the combination of characteristics needed in a broad range of assays in resource-limited environments. We
take the advantage of three facts: i) The market for blood testing devices is sufficiently large that the costs for development of this successful and robust technology have already been absorbed. ii) These electrochemical devices integrate smoothly with Micro-Paper-based Analytical Devices (µPADs)\(^6,13\) that we are developing, and can thus be adapted to analyze a broad range of analytes. iii) The output of these systems can be read directly, or coupled to cell phones, for telemedicine-based applications.

**EµPADs.** We\(^14\) and others\(^15-17\) have recently developed microfluidic paper-based electrochemical devices capable of quantitative analysis of a range of substances (e.g. glucose, enzymes, serum proteins, and heavy metal ions) in aqueous solutions. Although electrochemistry provides an enormously powerful set of analytical methods, electrochemical systems of the types used in research, industrial, and clinical laboratories are too expensive and cumbersome to be practical for use in resource-limited environments. Glucometers provide, we believe, a way to bridge the gap between laboratory use and field use of electrochemistry.

Commercially available electrochemical glucose test strips are typically made on a plastic substrate, and their price (including, of course, margin) in the U.S. — \(~\$0.5-1.0/strip\)\(^1,18\) — is impractically high for applications in the developing world. Test strips that would be produced at lower cost, and that would assay analytes other than glucose, might fit a range of uses. Glucose testing for diabetes control is currently the dominant application of glucometers, but we believe that this technology can be extended to a large group of substances other than glucose.

The electrochemical analytical system described in this article has at least five advantages: i) It offers a simple, fast (<60 s for detection, for systems that develop most
rapidly), and low-cost (at current stage of development, ~$0.014/strip for the materials and use of equipment for a glucose test). There are clear opportunities to lower this cost further, and to fabricate a glucometer for substantially less than $10. ii) The strips are light-weight, portable, rugged, and safely disposable by incineration. iii) It does not require professional medical personnel or complicated instruments. iv) The same reader can be adapted to a range of different analytes. v) Electrochemical methods are insensitive to light, dust, and insoluble particulates, and are thus applicable to dirty environments, and to samples containing suspended solids (where optical methods might fail). This system has three disadvantages: i) It requires batteries (one 3-V lithium battery is capable of carrying out approximately 1100 measurements). ii) Its assays are susceptible to interferences from electrochemically active substances. iii) Certain assays may be sensitive to temperature.

EXPERIMENTAL DESIGN

Fabrication of the Devices. We fabricated paper-based microfluidic channels by patterning chromatographic paper (Whatman 1 Chr) by wax printing, as described previously. We screen-printed wires and contact pads using silver ink, and four electrodes from graphite ink, on a piece of patterned paper. The external circuits do not contact the solution of analytes being measured, and less expensive conducting inks (e.g., copper or aluminum ink) could be used to lower the cost of the test strips further.

Glucometers. We chose the True Track blood glucometer (CVS/Pharmacy) as the electrochemical reader. This glucometer has two attractive characteristics: i) Its cost is low (the meter retails for about $20 for each; it is, however, usually supplied free with the test strips), and it is simple to use. ii) It is easy to reverse engineer the format used in
its test strips into a format that fits our needs. Other glucometers could, however, also be used. The design required differs with the test: one that requires concentration — for example, for the analysis of water — might be different from one that works with blood, and one that required removing cells from blood by filtration might be different from the one used with whole blood or urine.

Since commercial test strips may vary from batch to batch, this model of glucometer requires the user to enter a code on a code chip that comes with the test strips. Inserting the code chip into the glucometer calibrates it for that batch of test strips (Figure SI2). We have not tried to replicate this level of calibration in our present work.

**Design of Paper-based Electrochemical Devices.** We designed the EµPADs to fit into the port of the glucometer. We fabricated the circuits of the EµPAD to mimic the format of the test strips sold for this device. We treated the detection zones of the EµPAD with a solution of 2 wt% 3-aminopropyltrimethyl-ethoxysilane (APDES) in water to enhance the hydrophilicity of the paper channels, and of the electrodes.\textsuperscript{23,24}

**Methods and Principles of Detection.** We measured concentrations of D-glucose, cholesterol, L-lactate, and ethanol on the EµPADs using amperometry, utilizing the glucometer as an electrochemical reader. Eq. 2 generalizes the reactions for the amperometric detection of D-glucose, cholesterol, and L-lactate\textsuperscript{1,10,12,25}.

\[
\text{Substrate(CHOH or CH=O) + } H_2O \xrightarrow{\text{Enzyme}} \text{Substrate(CH=O or COO}^-\text{) + 2H}^+ \quad 2\text{Fe(III)CN_6}^{3-} \xrightarrow{2e^-} 2\text{Fe(II)CN_6}^{4-} \tag{2}
\]
In the enzyme-catalyzed step, glucose oxidase (GOx), or another oxidase, catalyzed the oxidation of glucose (or cholesterol or lactate) to gluconic acid (or cholest-4-en-3-one or pyruvate) with concomitant reduction of Fe(III) to Fe(II) \((\text{eq. } 2)\); The \(\text{Fe(II)(CN)}_6^{4-}\) ions generated were detected amperometrically.

\[
\text{Eq. 3 and 4 describe the mechanism of enzymatic detection of ethanol in the presence of } \beta-\text{NAD}^{+10}. \text{ The oxidation of ethanol to acetaldehyde in a reaction catalyzed by alcohol dehydrogenase reduced } \beta-\text{NAD}^+ \text{ to NADH (eq. 3). The electron-transfer mediator ferricyanide, present in the solution, rapidly oxidized the NADH to } \beta-\text{NAD}^+ \text{ with concomitant reduction of Fe(III) to Fe(II) (eq. 4); the } \text{Fe(II)(CN)}_6^{4-} \text{ ions generated were detected amperometrically.}
\]

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{OH} + \text{NAD}^+ & \xrightarrow{\text{Alcohol dehydrogenase}} \text{CH}_3\text{CHO} + \text{NADH} + \text{H}^+ \\
\text{NADH} & \xrightarrow{2\text{e}^-} \text{NAD}^+ + \text{H}^+ \\
2\text{Fe(III)CN}_6^{3-} & \rightarrow 2\text{Fe(II)CN}_6^{4-}
\end{align*}
\]

**RESULTS AND DISCUSSION**

**Optimization of the Design of \(\text{E}_\mu\text{PAD}\)**

We used \(\text{E}_\mu\text{PADs}\) made from a single layer of paper (rather than two or more layers, although multilayer designs may be useful, or even required, in some complex assays). These \(\text{E}_\mu\text{PADs}\) incorporated channels patterned with hydrophobic walls to control the flow of liquids and to support the electrochemical measurements (Figure 1A, B). A single-layer platform has at least three advantages: i) It allows reproducible conformal contact between the electrodes and the paper channels. ii) Its fabrication
can be scaled to large numbers ($10^{3-4}$ strips/day by hand or $10^{7-8}$ strips/day by machine). iii) It requires a smaller quantity of solution of analytes than multilayer devices.

It was important to treat the detection zone of the EµPADs with APDES or another agent that enhanced wetting, in order to: i) increase the hydrophilicity of the surface of the graphite electrodes, and thus the effective area of contact of the electrode surface with the solution of analytes (Figure SI3), and ii) increase the rate of wicking of the solution of analytes in the paper channel. If the rate of mass transport of fluids is not sufficiently rapid to deliver the fluids to wet all electrodes, the glucometer displays an error message.

**Evaluation of the Meter for the Use in EµPAD**

In order to fit the capability of the glucometer to our electrode geometry, we adjusted the dimensions of the electrodes of the EµPAD to make the measured currents fit the desired range of concentrations. Based on the Cottrell equation, the electrical current, $i$, of the system is linearly proportional to the surface area, $A$, of electrode ($i \propto A$). In principle, the same objective might be achieved by reprogramming the code chip that comes with the glucometer; but to do so would require more understanding of the design, circuiting, and control of the device than we have.

To evaluate the compatibility of the commercial glucometers with our EµPADs, we generated a calibration curve for the measurement of glucose in human plasma (Figure 2). The value of glucose concentration displayed by the glucometer increased linearly with the concentration of glucose, [glucose]; the slope of this plot was 1.09 units/(mg·dL$^{-1}$) (intercept, 17.0, correlation coefficient, 0.993). The analysis of glucose
**Figure 2.** Calibration plots for the analysis of glucose in human plasma in commercial test strips (○) and in EµPADs (●) made from a single layer of paper, using a commercial glucometer. The solid lines represent linear fits to experimental data with regression equations: \( y = -2.9 + 1.05x \) (\( R^2 = 0.997, n=7 \)) (○), and \( y = 17.0 + 1.09x \) (\( R^2 = 0.993, n=7 \)) (●). Inset shows the readings of glucose concentration in EµPADs plotted as a function of glucose concentration measured by commercial test strips. The solid line in the inset represents a linear fit to experimental data with a regression equation: \( y = 11.9 + 1.05x \) (\( R^2 = 0.995, n=7 \)). [Glucose]_paper: the displayed concentration of glucose in EµPADs. [Glucose]_commercial: the displayed concentration of glucose in commercial test strips.
Figure 2

The figure shows a calibration curve for glucose measurements, comparing displayed values to actual glucose concentrations. The x-axis represents glucose concentrations in millimoles (mM), while the y-axis shows displayed values. The inset graph further illustrates the relationship between commercial glucose concentrations and displayed values.
in human plasma in commercial plastic-based test strips produced a similar linear calibration curve with a slope of 1.05 unit/(mg⋅dL\(^{-1}\)) (intercept, -2.9, correlation coefficient, 0.997) (Figure 2).

The range of linear concentrations in E\(\mu\)PADs (from 0 to 500 mg/dL) covers the medically relevant range of glucose concentrations (~ 70-120 mg/dL).\(^1\) A wider linear range of concentrations — for use, for example, in food testing — could be achieved by optimizing the geometry of the device and the surface area of working electrode, although the precision of the device might suffer.

Table 1 summarizes the comparison between the performance of the analysis of glucose in E\(\mu\)PADs with that in commercial test strips. The limit of detection (LOD) was calculated as the concentrations which produced three times SD\(_0\), where SD\(_0\) is the value of the standard deviation as the concentration of the analyte approaches zero. The lower LOD of 26 mg/dL glucose obtained using E\(\mu\)PADs is slightly larger than the LOD of 15 mg/dL glucose achieved with commercial test strips. The mean coefficient of variation in these analyses in E\(\mu\)PADs was 9.1%. This value is approximately twice the 4.1% of the commercial glucose test strips.\(^2\) We attribute the higher value of this coefficient of variation in E\(\mu\)PADs to variations in the width of paper channels fabricated by wax printing, and to variations in the width of electrodes fabricated by screen printing; the reproducibility of measurements in our system could certainly be increased by improved engineering and standardized fabrication.

The minimum volume of samples required to wet the paper channel completely was approximately 1.0 \(\mu\)L for the design of the E\(\mu\)PAD used for this specific assay; this quantity can be decreased further by shortening the length of paper channel, or by using a
Table 1. Comparison of the Performance of EµPADs with Commercial Plastic-based Glucose Test Strips.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Linear dynamic range (mg/dL)</th>
<th>Limit of detection (mg/dL)</th>
<th>Mean coefficient of variation (%)</th>
<th>Minimum volume of sample (µL)</th>
<th>Test for blood samples (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial test strips</td>
<td>~0 – 550&lt;sup&gt;a&lt;/sup&gt;</td>
<td>~26</td>
<td>4.1</td>
<td>~1.0</td>
<td>99±3&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Paper strips</td>
<td>~0 – 500</td>
<td>~15</td>
<td>9.1</td>
<td>~1.0</td>
<td>95±9&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> The linear dynamic range reported previously was 0 – 600 mg/dL.<sup>22</sup> <sup>b</sup> The mean coefficient of variation was calculated by averaging relative standard deviations of the measurements of standard solutions with glucose concentration of 25, 50, 100, 150, 200, 300, 400, and 500 mg/dL.<sup>c</sup> For the analysis of blood samples with unknown concentration of glucose, a small drop of blood was obtained by pricking the skin with a steel lancet.<sup>d</sup> Three measurements were averaged. <sup>e</sup> Eight measurements were averaged.
thinner paper. This EµPAD is probably sensitive to temperature, due to rates of evaporation and wicking of solutions.

We compared the analysis of glucose in human whole blood using EµPADs with commercial test strips. We brought the inlet of an EµPAD into contact with a small droplet of blood obtained from a fingerprick. The blood, containing blood cells, rapidly filled the paper channel by wicking (this way does not require the paper to remove cells), and the glucometer initiated the electrochemical measurement, and displayed the result of measurement. The levels of glucose in whole blood are generally 10-15% lower than glucose in plasma, and the concentrations of glucose in blood, [Glucose]blood, can be approximated to the measured values of plasma glucose, [Glucose]plasma ([Glucose]blood=[Glucose]plasma/1.14). The calibration curves for the analysis of glucose in human plasma (Figure 2) were therefore, used to determine the concentration of glucose in blood. The corrected concentration of glucose in blood was $95 \pm 9$ mg/dL (n=8) measured in EµPADs; this value was about 4.4% lower than the value $99 \pm 3$ mg/dL (n=3) obtained in commercial test strips.

We conclude that — at this stage of the work (laboratory prototype) — the performance of the EµPADs is roughly equivalent to that of commercial test strips. Since we are using the same chemistry as that used commercially, the agreement is not surprising. It does, however, validate EµPADs as electrochemical sensors for use — in conjunction with commercial glucometers — in biomedical sensing.

Applications in Analyzes Other Than Glucose

We evaluated the feasibility of using EµPADs and this glucometer to measure
the concentration of analytes other than glucose. We demonstrated the analysis of cholesterol and L-lactate in human plasma as well as ethanol in aqueous solutions.

**Clinical Diagnostics: Analysis of Cholesterol and L-Lactate in Body Fluids**

The concentration of cholesterol in human plasma is less than 5.2 mM (200 mg/dL).\(^{30}\) The analysis of cholesterol in human plasma using cholesterol oxidase yielded a linear calibration plot in the concentrations ranging from 20-200 mg/dL (0.5-5.2 mM);\(^{31}\) these values cover the clinically relevant range of cholesterol concentrations (Figure 3A).\(^{30}\) The limit of detection was 13 mg/dL (0.34 mM), and the sensitivity was approximately 0.8 unit/(mg·dL\(^{-1}\)). The mean coefficient of variation of these analyses was about 6.2% (n=7).

The clinically relevant range of L-lactate concentrations is from 0.5 to 15-20 mM in serum or plasma.\(^{32,33}\) Commercial lactate meters have a range of 0.8–23.3 mM with a 60 s sampling time, and require 5 µL of sample. We demonstrated the use of the glucometer to analyze the concentration of L-lactate in human plasma. The calibration curve for the measurement of L-lactate shows that the values displayed are linearly proportional to the L-lactate concentrations in the range of 1-11 mM with a sensitivity of 2.8 units/(mg·dL\(^{-1}\)) (~ 25.5 units/mM) (Figure 3B). The concentration range for quantitative detection is, therefore, slightly narrower than the clinically relevant range at this stage of development of EµPADs. The reliable lower limit of detection was 9.8 mg/dL (~1.1 mM).\(^{34}\) Both assays of cholesterol and L-lactate on these specific EµPADs require approximately 1.2-1.5 µL of fluid; this value is determined by the quantity required to wet the paper channels completely.

Although it would be straightforward to tune the geometry of EµPADs to
Figure 3. Application of glucometers as electrochemical readers for measuring the concentrations of cholesterol and L-lactate in human plasma. Calibration plots for the analysis of cholesterol (A) and L-lactate (B) in EµPADs. The solid lines represent linear fits to experimental data with regression equations: (A) $y=90.1+0.8x$ ($R^2=0.962$, n=7), and (B) $y=236.7+2.8x$ ($R^2=0.989$, n=7).
Figure 3

A) 

B)
adjust the displayed values closer to actual concentrations of analytes, we have not
done so for the cholesterol and L-lactate systems in the same way as we did for the
glucose system. It would also be possible to use a chip to adjust for the response
produced by our EµPADs, since different batches of commercial test strips are
accompanied with individual calibration codes embedded in code chips.

We note that the glucometer displays a non-zero value in the analysis of
sample solutions in the absence of analytes (Figure 3). We attribute this value to
background contributions due to the charging process of the electrical double layer,
and to the redox reactions of ferrocyanide generated by the degradation of a small
fraction of ferricyanide in solutions (even if the solution of electron-transfer
mediators is freshly prepared). This measured value could, however, be calibrated to
display an actual concentration of analytes.

**Food Quality Control: Analysis of Alcohol in Water** The electrochemical
system has the potential to be useful in food quality control. We used EµPADs and
glucometer to measure the concentration of ethanol. The calibration plot for the
analysis of ethanol (Figure 4A, B) showed a linear range from 0.1 to 3 mM
\( (R^2=0.970) \) with a sensitivity of 54 units/mM. The limit of detection was 0.1 mM, and
the coefficient of variation ranged from 3.2% to 10.1%.

Table 2 summarizes the performance of this electrochemical system for these
analyses. The linear ranges of detection as well as the limit of detection achieved in
EµPADs cover useful ranges, but leave substantial room for engineering
improvement.

**CONCLUSIONS**
The EµPADs are compatible with commercially available glucometers. The use of glucometers as readers for EµPADs substantially increases the range of options for combining paper–based analytical devices and electrochemical detection for applications in resource-constrained environments. This electrochemical system has six potential advantages: i) It is portable, reliable, and inexpensive. ii) It takes advantage of the sophisticated engineering already embedded in commercially available, inexpensive glucometers. iii) It can be adapted to analytes other than glucose. iv) Electrochemistry — unlike colorimetry and spectrophotometry — is insensitive to local light conditions, and to certain types of contaminants (suspended solids, colored materials) present in samples. v) It can be interfaced with a cell phone (either by human reporting of the data, by photography the LCD display, or, in principle by a coded interface). It can also be used in home patient care with telephone or internet communications. vi) It can also, in principle, be adapted to a range of different types of assays (amperometry, as here; chronoamperometry, cyclic voltammetry, annodic stripping voltammetry, electrochemiluminescence, and others).

The EµPAD has at least five useful characteristics for applications in resource-limited environments (no plastic strips have all of these characteristics): i) It is well suited to mass-production at low cost using wax-printing and screen-printing technologies. It can, therefore, be manufactured almost everywhere, and easily adapted to local use. ii) It can be used in analyses of complex fluids (e.g., blood, urine, and saliva), even when they contain suspended solids and dirt. iii) Its fundamental design is sufficiently simple that this design can be modified to generate test strips that fit into different types of glucometers, or that carry out different
Figure 4. Glucometers as electrochemical readers for the analysis of ethanol. (A) Calibration plot for the analysis of ethanol in aqueous solutions, and (B) an enlarged graph in the dashed square in (A). The solid line in (B) represents a linear fit to experimental data with a regression equation: \( y = 139.4 + 53.9x \) \((R^2 = 0.970, n=7)\).
Figure 4

A)

B)
Table 2. Glucometers as Electrochemical Readers for Amperometric Detection in EµPADs.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Enzyme</th>
<th>Electron-transfer mediator</th>
<th>Dynamic linear range</th>
<th>Limit of detection</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>Glucose oxidase</td>
<td>ferricyanide</td>
<td>0-500 mg/dL</td>
<td>26 mg/dL</td>
<td>pre-stored&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Alcohol dehydrogenase/β-NAD&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ferricyanide</td>
<td>0.1-3 mM</td>
<td>0.2 mM</td>
<td>pre-stored</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Cholesterol oxidase</td>
<td>ferricyanide</td>
<td>20-200 mg/dL</td>
<td>13 mg/dL</td>
<td>pre-mixed&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>L-lactate</td>
<td>Lactate oxidase</td>
<td>ferricyanide</td>
<td>1.1-11 mM</td>
<td>1.1 mM</td>
<td>pre-mixed</td>
</tr>
</tbody>
</table>

<sup>a</sup> Pre-stored: we stored chemical reagents needed for the assay on the EµPAD, and carry out the assay with the EµPAD in glucometer.  <sup>b</sup> Pre-mixed: we mixed chemical reagents needed for the assay with a solution containing analytes, and allowed the reaction to proceed to completion off the EµPAD. The glucometer was used simply as an amperometer to read the result.  

The commercially available human plasma itself contains 1.1 mM lactate before the addition of any lactate. In fact, we were able to detect 0.5 mM of lactate in PBS buffer solution (pH 7.0).
analytes. iv) It is based on a system of paper-based microfluidic devices of rapidly increasing sophistication. The programming of flows of fluids in these devices makes it possible to carry out analysis requiring multiple steps, without using extra instruments such as pumps.\textsuperscript{36} v) It has sufficient flexibility to incorporate a variety of different functionalities — concentration of analytes by evaporative heating, and chromatographic separation of analytes from interference — while retaining the interface to the glucometer.

At this stage of development, the E\textsubscript{µ}PADs that we have fabricated are slightly less accurate than commercial test strips, possibly due to the manual procedures we used in their fabrication; this accuracy will certainly improve with further engineering and attention to manufacturing detail.

**ACKNOWLEDGEMENTS**

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18. The production of each test strip costs about $0.05-0.15.

19. Silver ink 250 g costs about $150, and one g of ink can produce about fifty devices by manual screen printing; silver ink for one device costs $0.012. Graphite ink 1000 g costs about $200, and one g of graphite ink can produce about two hundred devices by manual screen printing; graphite ink for one device costs $0.001. One page wax printed paper (~ 200 devices) costs about 20 cents; wax printing for one device costs $0.001. Thus, one device costs $0.014 in total. We found that the lowest price for copper ink is $75/1000 g. The cost of one device based on copper ink is $0.0035. The estimation above is based on the price of commercial products.

20. Ferricyanide emits toxic fumes of cyanides and oxides of nitrogen when heated to decomposition; it, however, would not cause a problem when such action is away from human beings and animals. We aware that other cheap non-toxic electron transfer mediators can certainly be used.


23. We attribute the improved wettability of surface in contact with electrolyte to the introduction of amino groups onto the surface.


26. We did not observe an obvious influence of the length of screen-printed wires and contact pads on the electrochemical readout using the glucometers.


28. The average hematocrit in an adult constitutes about 45% of whole blood by volume, and the plasma about 55%. The water content in the plasma and red blood cells by volume was approximately 92% and 70%, respectively. Thus, the water content by volume in whole blood and plasma was approximately 82% and 92%, respectively. As glucose is passively transported through the plasma membrane of erythrocytes, the levels of glucose in whole blood are generally 10-15% lower than glucose measurements in plasma.


31. Artificial human plasma was used for these specific assays of cholesterol due to the presence of large amounts of cholesterol in human plasma (or whole blood) purchased from Innovative Research, Inc. (http://www.innov-research.com/innov2010/).


34. The commercially available human plasma itself contains 1.1 mM lactate before the addition of any lactate. In fact, we were able to detect 0.5 mM of lactate in PBS buffer solution (pH 7.0).

