

Electrochemical Assay System with Single-Use Electrode Strip for Measuring Lactate in Whole Blood

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We have developed an assay system for measuring lactate in whole blood, consisting of a single-use strip of an enzyme-coated electrode and a small meter. The electrode strip is made of three plastic films: a cover sheet, a spacer, and an insulation layer printed with electrodes that are coated with lactate oxidase (EC 1.1.3.x) and ferricyanide as an electron mediator. The meter measures the magnitude of the anodic current of the reduced mediator by the enzymatic reaction and displays the lactate concentration 60 s after a blood sample (5 μ L) is applied. The calibration curve was linear up to 20 mmol/L, and the between-run CVs at three concentrations were 1.7-8.4%. Lactate concentrations determined by this method (y) in blood samples from healthy individuals before and after exercise agreed with the results obtained by the conventional enzymatic method (x): $y = 0.97x - 0.3$, $S_{y/x} = 0.7$. This assay provides a rapid and convenient test for measuring blood lactate concentrations.

Indexing Terms: enzymatic method · point-of-care testing · lactate oxidase

Lactate concentration in blood is a biochemical indicator of anaerobic metabolism in patients with circulatory failure. When the blood flow is markedly decreased, oxygen delivery is no longer adequate to sustain aerobic metabolism, creating an oxygen debt. The concentration of lactate in blood corresponds to the extent of the oxygen deficit, and measurement of blood lactate gives information for diagnosis and estimation of a prognosis in affected patients (1). Blood lactate concentrations increase during physical exercise, and the changes in these concentrations are useful for estimating physical fitness and the effects of exercise (2, 3). An electrode analyzer with an enzymatic sensor can measure lactate concentrations in whole blood and overcomes the limitations of conventional enzymatic assays, which are time-consuming and technically complex (4, 5). We developed an assay system for measuring lactate concentrations in whole blood that includes a single-use strip of an enzyme-coated electrode and a small meter containing a data processor. We used this system to study blood samples from healthy individuals before and after exercise, and here we report our results.

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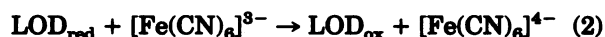
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Materials and Methods

Reaction principle. The following schema, where LOD_{ox} and LOD_{red} represent oxidized and reduced lactate oxidase, respectively, describes the reaction sequence.



Reactions 1 and 2 occur simultaneously. Lactate in the blood is specifically oxidized by LOD_{ox} to pyruvate; LOD_{ox} is reduced to LOD_{red} . LOD_{red} is oxidized with ferricyanide, an electron mediator, to LOD_{ox} , which again oxidizes lactate. Thus LOD repeatedly oxidizes lactate and changes the mediator to the reduced form, ferrocyanide. The accumulated ferrocyanide is finally oxidized to ferricyanide, as shown in equation 3, by an electrode with a potential of +0.5 V. The anodic current is measured by an amperometer.

Electrode strip. A 24 \times 6 mm strip was made of three plastic films: a cover sheet, a spacer, and an insulation layer. A reaction layer was formed on the carbon electrodes, which were printed on the insulation layer (Figure 1). The reaction layer was cast and dried from 2 μ L of 5 g/L carboxymethyl cellulose (Dai-ichi Kogyo Seiyaku Co., Kyoto, Japan) containing 1.6 U of lactate oxidase (EC 1.1.3.x) from *Pediococcus sp.* (Asahi Chemical Industry Co., Shizuoka, Japan) and 50 mmol of tripotassium hexacyanoferrate (Nacalai Tesque, Kyoto, Japan). About 5 μ L of whole blood aspirated by capillary action into the open end of the spacer moved into the space between the three films.

Meter. The Glucocard[®] blood glucose meter (Kyoto Dai-ichi Kagaku Co., Kyoto, Japan) (6) was modified for lactate measurements. The potential (+0.5 V) was ap-

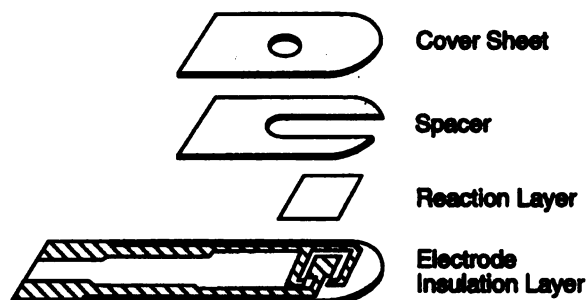


Fig. 1. Schematic of the single-use electrode strip

plied 55 s after sample introduction, and the magnitude of the anodic current was measured instantaneously by the meter. The meter was powered by two lithium batteries. The size of the meter was 86 × 53 × 13 mm; its weight was 50 g. The data processor in the meter calculated the lactate concentration from the current by using a programmed calibration curve, and the value was displayed. The meter has a thermosensor and a program to decrease the effect of temperature on measurements.

Procedure. An electrode strip was inserted into the strip inlet of the meter, and the meter switched on automatically. When the other end of the strip touched a drop of whole blood, about 5 μ L was aspirated and measurement started. The lactate concentration was displayed after 60 s; the meter turned off when the strip was removed. The used strip was discarded.

Comparison method. We also measured lactate concentrations in blood samples with a kit (Determiner LA; Kyowa Medix, Tokyo, Japan). The analysis was based on the conversion of lactate to pyruvate and hydrogen peroxide by lactate oxidase in the presence of oxygen. The generated hydrogen peroxide reacts with the chromogens 4-aminoantipyrine and *N*-ethyl-*N*-(3-methylphenyl)-*N'*-acetyl ethylenediamine, catalyzed by horseradish peroxidase. Absorbance was measured at 415/550 nm with a Hitachi 7150 analyzer (Hitachi, Tokyo, Japan).

Blood sampling. Blood samples were obtained from 10 healthy volunteers before, during, and after exercise on an ergometer, and the workload was increased gradually until the individuals reached exhaustion. Before beginning the study, we obtained informed consent from all participants. Immediately after blood sampling, we assayed the concentrations of lactate with our system; another portion of the blood sample was treated with perchloric acid and assayed by the comparison method.

Results

The lower limit of detection by this new method was 0.1 mmol/L. The calibration curves for lactate concentrations in whole blood and plasma were linear up to 20 mmol/L (Figure 2). Table 1 shows that the assay precision

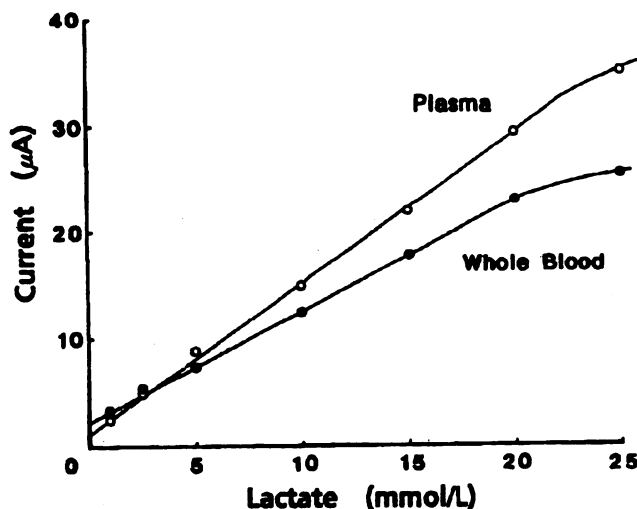


Fig. 2. Calibration curves for lactate in whole blood and plasma

Table 1. Precision of the Electrode Strip Method

	Mean \pm SD, mmol/L	CV, %
Sample A	0.94 \pm 0.08	8.35
Sample B	3.50 \pm 0.06	1.67
Sample C	8.63 \pm 0.23	2.65
n = 10 each.		

for blood samples with lactate, assayed 10 times each, is 1.7–8.4% (CV). During this procedure, sodium fluoride was added (24 mmol/L) to inhibit lactate production; this concentration had no effect on the assay procedure and was sufficient to inhibit the increase in blood lactate for 3 h. Heparin was added as an anticoagulant. Glucose at a final concentration of 44.4 mmol/L, creatinine at 884 μ mol/L, uric acid at 1190 μ mol/L, bilirubin at 137 μ mol/L, glutathione (reduced form) at 1.3 mmol/L, acetaminophen at 1.0 mmol/L, sodium salicylate at 2.0 mmol/L, glycerate at 2.0 mmol/L, and 3-hydroxybutyrate at 3.5 mmol/L had a negligible effect on the results of the blood lactate measurement. Addition of ascorbic acid at a final concentration of 1.14 mmol/L caused a 37% increase in the results.

We examined the effect of hematocrit on the assay by measuring lactate concentrations in samples with different hematocrit values (Figure 3). The samples were prepared by separating plasma and erythrocytes in the presence of heparin and sodium fluoride, then mixing them again to produce the desired hematocrit. In sample 1, with a high lactate concentration, the lactate values in the low-hematocrit samples were higher than those in a normal-hematocrit sample; the lactate values in samples with a high hematocrit were even lower. In sample 2, with a low lactate concentration, the measured values were little affected by differences in hematocrit. The correlation between lactate concentrations in 80 blood samples from the individuals before, during, and after exercise, measured by the electrode strip method (y) and by the comparison method (x), which is the usual method in Japan, was $r = 0.976$ ($y = 0.966x - 0.254$ mmol/L, $S_{y|x} = 0.678$ mmol/L).

Discussion

An electrode analyzer with an enzymatic sensor that can measure lactate concentrations directly in whole

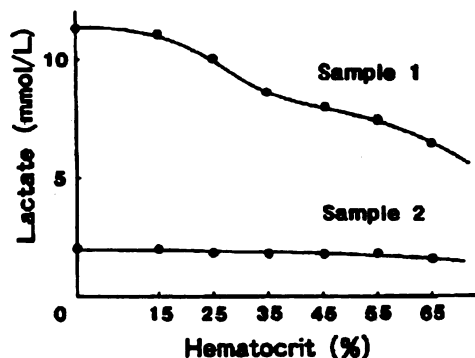


Fig. 3. Effect of hematocrit on measured lactate values. Two blood samples with different lactate concentrations were assayed by the procedure described in the text.

blood offers the advantage of easy handling of samples without the need for immediate deproteinization and centrifugation (4, 5). The test-strip method also provides rapid and precise measurements of lactate concentrations in whole blood (7). An amperometric assay of blood glucose with a ferrocene-mediated enzyme electrode has been developed and used to assay blood glucose with a single-use electrode strip (6, 8, 9). The conventional meters for glucose self-monitoring have glucose oxidase strips that change color. The disadvantages of these systems are that the user has to perform the test in a precisely timed cycle, and the blood-stained end of the strip that is inserted into the meter may contaminate it and cause inaccurate measurements. The assay system for measuring blood glucose with a single-use electrode strip resolves these issues.

Our assay system for measuring blood lactate also has a single-use electrode strip. The calibration curve for blood lactate was linear up to 20 mmol/L, which exceeded the maximum concentrations of blood lactate after exhaustive exercise. The required sample volume was 5 μ L of whole blood, which seems particularly appropriate for use in obstetric and neonatal intensive care. In sports medicine, the use of a lancet for blood sampling makes it possible for coaches or athletes to measure blood lactate concentrations without the help of a doctor or a nurse. The new method is easy to operate, and the results are available in 60 s. The correlation between the lactate concentrations measured with the strip and the kit was high. In samples with a high lactate concentration, the lower the hematocrit, the higher the displayed lactate concentration. This result suggested that lactate concentrations in plasma samples could not be measured with this system. However,

we observed a linear relation between the lactate concentrations in plasma and the magnitude of the electric current generated (Figure 2). The correct lactate concentrations in plasma were obtained when the concentrations were calculated with another calibration curve for plasma lactate measurements.

The new assay system is a rapid, convenient, and reliable method for measuring lactate concentrations in whole blood, and the meter itself is pocket-sized. These characteristics allow lactate to be measured at the bedside, in the laboratory, and even outdoors for application in clinical and sports medicine.

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Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis of Urine: Concentration of Urinary Proteins by Precipitation with Coomassie Blue

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The precipitation of urinary proteins by Coomassie blue (*Clin Chem* 1992;38:1186-7) has been exploited for protein concentration prior to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Making the electrophoresed proteins visible by Coomassie blue staining confirms concentration and reveals ≤ 40 polypeptide bands in urine of normal protein content and distribution.

Making the electrophoresed proteins visible by silver staining confirms good protein recovery and suggests little modification of the electrophoretic profile. Excess dye can impair resolubilization of the protein and adversely affect electrophoresis, and should be removed by an acetone wash. The residual dye completely dissociates from the protein and can be used as a dye front during electrophoresis. The method combines protein assay and recovery and is particularly suited to high sample throughput. Concentration is simple, rapid (<30 min), and economic, but the choice of dye reagent is important because some commercial reagents are designed to enhance solubility of the protein-dye complex.

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