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Improving Lactate Analysis with the YSI 2300 GL: Hemolyzing Blood Samples Makes Results Comparable with Those for Deproteinized Whole Blood

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To obviate the well-documented problem of hematocrit dependency of the Yellow Springs Instruments (YSI) whole-blood lactate analyzer, we modified the dilution buffer by including a lysing reagent. This makes the results comparable with those of methods performed with deproteinized whole-blood samples. No centrifugation step is needed, thus preserving the convenience of the YSI instrument for stat and field use. The modification works equally well on plasma samples. Lactate concentrations measured in nonhemolyzed whole blood are not comparable with results for hemolyzed whole blood or protein-precipitated whole blood. We therefore recommend to all users of YSI equipment to lyse the erythrocytes before lactate determinations.

Additional Keyphrases: analytical error • hematocrit • enzyme electrodes • sample treatment

To facilitate rapid measurements of blood lactate, Yellow Springs Instruments (YSI; Yellow Springs, OH) developed an instrument based on membrane-bound enzyme electrode methodology. Clark et al. (1) found it rapid, accurate, and reliable, and the method is popular in clinical settings and has been used in several studies. However, the YSI instrument measures isotonically diluted whole blood (extracellular lactate). As indicated by Weil et al. (2) and Wandrup et al. (3), the results obtained differ from measurements of deproteinized whole-blood samples (both extra- and intracellular lactate), which is the normal sample treatment before analysis by standard comparison methods (spectrophotometric enzymatic substrate measurements). Because erythrocytes contain ~50% less lactate per volume than does plasma (4–6), the difference between the YSI method and the comparison methods is hematocrit dependent.

Instead of using a calculation formula based on tedious hematocrit measurements and the measured whole-blood lactate value to derive the total blood lactate concentration, we developed a simple sample treatment that includes a hemolyzing agent in the YSI buffer.

Materials and Methods

Reagents

The buffer reagent kit from YSI contained sodium dihydrogen phosphate (25 mmol/L), sodium hydrogen phosphate (45 mmol/L), and sodium chloride (31 mmol/L). The immobilized L-lactate oxidase (EC 1.1.1.27) enzymatic kit was obtained from YSI. L-Lactate (1.0 mol/L) and Peroxidase (Boehringer Mannheim, Frankfurt, FRG) were used for the calibration and quality-control material, respectively.

Equipment

We used the Model 2300 GL lactate analyzer from YSI. The instrument utilizes an electrode technology in which L-lactate oxidase is immobilized in a thin membrane placed over an electrochemical probe. The enzyme catalyzes the conversion of L-lactate to pyruvate and hydrogen peroxide, the latter then being oxidized at the platinum anode. A stainless-steel electrode completes the circuits, and a silver chloride electrode is used as the reference electrode.
Blood Samples

Venous and capillary blood samples were taken from apparently healthy volunteers at rest and during exercise.

Procedures

Comparison method. A Multistat III centrifugal analyzer and a Multistat III loader (Instrumentation Laboratory, Lexington, MA) were used in combination with a Monotest lactate kit (Boehringer Mannheim), as previously described by von Schwab et al. (7).

Modification of YSI method. Triton X-100 (Technicon, Tarrytown, NY) was added to the YSI buffer in a concentration of 20 g/L. We used this buffer for sample dilution as well as for the system buffer in the instrument. We saw no interference in the electrode reaction by including the lysing reagent Triton X-100 up to a concentration of 50 g/L in the YSI original buffer solution.

Initial trial. We evaluated the electrode linearity by using the YSI method to measure a serially diluted 1.0 mol/L L-lactate standard. Venous whole blood was collected in Vacutainer Tubes containing heparin and NaF (Becton Dickinson, Rutherford, NJ). We deproteinized 100 μL of whole blood by precipitation with 900 μL of perchloric acid (0.3 mol/L) before determining the total blood lactate concentration by the comparison method. Plasma was analyzed by the YSI method and by the comparison method. The erythrocyte volume fraction (EVF) was determined by centrifugation. One proposed formula from Wandrup et al. (3) [i.e., \(LP = \frac{LB}{1 - 0.88 \times EVF}\)] for converting whole-blood lactate concentration (LB) to plasma lactate concentration (LP) was also evaluated by comparison with the measured plasma lactate concentration.

To evaluate the optimal concentration for hemolyzing the erythrocytes, and to prevent clotting in the instrument pipettor, we added Triton X-100 to YSI original buffer, to give concentrations between 0.25 and 25 g/L. We then diluted the whole-blood samples with an equal volume of the buffer and gently stirred. The turbidity caused by nonhemolyzed erythrocytes was detected by absorbance measurement with a conventional photometer at 600 and 700 nm within 2 min after dilution. An absorbance <0.05 A was considered as complete hemolysis.

Main trial. We deproteinized 50 μL of whole blood with 450 μL of perchloric acid (0.3 mol/L). After centrifugation, we determined the lactate concentration in the supernate, using the comparison method. Whole blood was diluted with an equal volume of the lysing reagent (Triton X-100, 20 g/L, in YSI original buffer). After mixing this for 15–20 s, we determined the lactate concentration with the YSI instrument.

The comparison method and the YSI method were standardized with the same L-lactate standard, handled in the same way as the samples.

Analytical Variables

EVF. The effect of EVF on the lactate concentration, as measured by the YSI instrument with the original and modified method, was tested by adding erythrocytes to plasma to give 10 different proportions of erythrocyte content. Each proportion was then diluted with an equal volume of either the lysing reagent (modified method) or isotonic saline (original method) and then analyzed with the YSI instrument within 10 min. EVF and the erythrocyte count were also measured in the different samples by an automated hematological instrument (H-6010; Technicon).

Stability of samples. Venous whole blood was collected in Vacutainer Tubes containing EDTA or EDTA + NaF and diluted with an equal volume of the following solutions: (a) EDTA blood with NaCl, 154 mmol/L (EDTA + saline); (b) EDTA + NaF blood with NaCl, 154 mmol/L (sodium fluoride); or (c) EDTA blood with YSI buffer containing various concentrations of Triton X-100 (Triton 0.25–20 g/L). The samples were then stored at 23 and 4 °C, and the lactate concentration was determined with the YSI instrument after 5 min to 24 h.

Whole-blood samples at three different lactate concentrations were diluted with an equal volume of the lysing reagent and then stored at 21, 4, and −18 °C. Each sample was analyzed daily for 32 days.

Imprecision. Plasma samples of low, medium, and high lactate concentration as well as Precinorm S were divided into 100-μL portions and kept frozen at −18 °C for 10 days. The lactate concentration was then analyzed with the YSI instrument after dilution with lysing reagent to determine the within-series, within-day, and between-day imprecision.

Analytical range. A 1 mol/L L-lactate standard was serially diluted to 0.1 mmol/L. Each dilution of standard was then analyzed in duplicate with the YSI instrument.

Carryover. L-Lactate was added to two whole-blood samples to final concentrations of 51 and 1.0 mmol/L, respectively. The samples were then analyzed six times in alternating order.

Standard addition. To EDTA blood, plasma, and saline we added lactate standard in concentrations varying from 0.5 to 10 mmol/L. Each sample was then analyzed in duplicate according to the YSI original sample-handling procedure and the modified procedure with Triton X-100 in the buffer.

Results

Electrode linearity. The electrode probe current (nA) was ~50% lower with the modified method than with the original method. However, the probe current was linearly related to lactate concentration (0.5–14.0 mmol/L), and there was no difference in correlation whether the original method \((r = 0.999)\) or the modified method \((r = 0.999)\) was used.

Hemolysis reagent. The 20 g/L concentration of Triton X-100 in buffer solution (10 g of Triton X-100 per liter of sample solution) was more than sufficient for complete hemolysis of the erythrocytes (Figure 1).

Whole-blood analysis. There was a high correlation \((r = 0.994)\) between the hemolyzed blood and the whole-blood lactate concentration determined with the YSI
original method, but the whole-blood lactate concentration was 25% lower by the YSI original method (Figure 2). A similar result (30% lower) was also seen when precipitated whole blood, analyzed by the comparison method (x), was compared with whole blood analyzed by the YSI original method (y): \( y = 0.03 + 0.70x \) \((r = 0.992, n = 57)\).

**Evaluation of YSI converting formula.** There was no significant difference between calculated [by the formula of Wandrup et al. (3)] and measured plasma lactate concentration.

Accuracy. There was a high correlation \((r = 0.994)\) and no significant difference \((y = 0.18 + 1.04x)\) between the precipitated blood lactate concentration measured by the comparison method and the hemolyzed blood lactate concentration measured by the YSI modified method. There was also no significant difference in plasma lactate concentration between the three different sample preparation methods (YSI original method, YSI modified method, and comparison method). There was a negative correlation between EVF and lactate concentration in hemolyzed samples and in whole-blood samples. However, the slope for the whole-blood determinations was steeper than the slope for the hemolyzed blood samples at the same EVF (Figure 3).

**Sample stability.** There was an increase of lactate concentration with increasing storage time in samples without Triton X-100 and in samples lysed with Triton X-100 at concentrations <2.5 g/L in buffer solution (Figure 4). Lactate concentration in hemolyzed blood (Triton X-100, 20 g/L buffer) was stable for 24 h at 23 °C and for 14 days at 4 °C (Figure 5). There was no change...
in lactate concentration for 32 days when samples were stored at −18 °C (data not shown).

Imprecision. The within-series imprecision was 0.8–3.3%, the within-day imprecision was 1.2–3.7%, and the between-day imprecision was 1.6–4.1%, depending on the lactate concentration (Table 1).

Analytical range. The lowest and highest lactate concentration measured with a within-series imprecision of 4% was 0.4 and 14 mmol/L, respectively. Samples with a lactate concentration >14.7 mmol/L must be diluted before measurement.

Carryover. There was no measurable carryover.

Standard addition. The slope for the added lactate concentration in whole blood was 20% lower than for standard, plasma, and hemolyzed whole blood (Figure 6).

Practical experiences. The mean lifetime of the enzyme membranes was 4–5 weeks; during that time, we analyzed ~1100 samples, for a very low enzyme cost per sample of US$0.06. The stability of the Triton X-100-hemolyzed samples made the method robust. Furthermore, we could design tests for measuring lactate in exercising subjects, which required a greater sampling frequency than the analytical capacity of the instrument.

Discussion

This study confirms that analysis for lactate by using the YSI 2300 GL instrument gives results comparable with those of the comparison method for plasma or deproteinized whole blood. However, the discrepancy between the nonhemolyzed whole blood and hemolyzed whole blood confirms the findings from Wandrup et al. (3), who showed that the YSI instrument measures only the extracellular lactate when the erythrocytes are intact. This discrepancy has been shown to be EVF dependent. However, the results from the EVF and the standard addition in vitro experiment do not explain the entire discrepancy of results for samples obtained during exercise. The excess difference is probably caused by parallel changes of pH, pO2, pCO2, and bicarbonate in the subjects’ plasma during exercise. These changes were shown earlier to influence the lactate exchange between plasma and erythrocytes (8, 9).

To avoid the result of the exchange between plasma and erythrocytes during preinstrumental sample preparation, we consider it more accurate to hemolyze the samples and thus secure the validity of the analysis.

In conclusion: The YSI 2300 GL lactate analyzer provides accurate and precise analyses of lactate concentration in plasma and whole-blood samples hemolyzed with the modified buffer. We do not recommend analyzing for lactate in nonhemolyzed whole-blood samples.

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References

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Cathepsin D Concentration in Breast Cancer Cytosols: Correlation with Disease-Free Interval and Overall Survival

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Cathepsin D (CD) is an aspartyl protease implicated in cancer metastasis. In this study of 331 patients, we show that patients with primary breast carcinomas containing high concentrations of CD have a significantly shorter disease-free interval (chi-square = 4.28, P < 0.05) and overall survival (chi-square = 7.7, P < 0.01) than patients with low concentrations. CD as a prognostic marker for overall survival was equally valuable for women younger (chi-square = 4.39, P < 0.05) and older (chi-square = 3.97, P < 0.05) than patients with low concentrations. CD as a prognostic marker for overall survival was also significant in patients with high concentrations of CD in the estradiol receptor (ER)-positive subgroup of patients (chi-square = 5.7, P < 0.025), but not in the ER-negative subgroup. Patients with tumors containing high concentrations of CD and low concentrations of ER had shorter disease-free intervals (chi-square = 15.1, P < 0.001) and lower overall survival (chi-square = 20.9, P < 0.001) than patients with high concentrations of ER but low concentrations of CD.

Additional Keyphrases: protease · enzyme activity · tumor markers · immunoradiometric assay

Cathepsin D (CD) is an aspartyl protease normally found in lysosomes (for a review, see ref. 1). It is induced by estradiol in certain estrogen receptor (ER)-positive breast cancer cell lines, but is produced constitutively by ER-negative cell lines (1). Recently, it was shown that overexpression of transfected cDNA for CD in transformed cells increased the malignant phenotype and metastatic potential (2). This finding and the report showing that CD can catalyze degradation of the extracellular matrix (3) suggest that CD may play a role in cancer metastasis.

In human breast cancer, high concentrations of CD were shown to be a marker for poor prognosis (4–7). We previously showed that high concentrations of CD in breast cancer cytosols correlated significantly with overall survival but not with disease-free interval (7). We then extended this study to include more patients and longer follow-up. We now show that high concentrations of CD correlate not only with survival but also with disease-free interval. In this investigation, we also assessed the value of CD as a prognostic marker in different subgroups of breast cancer patients.

Materials and Methods

Breast tumors were rapidly cooled in liquid nitrogen and then stored at −70 °C. The tumors were homogenized in 50 mmol/L Tris-HCl (pH 7.4) as previously described (7). CD was assayed with an immunoradiometric assay (ELISA-cath-D kits; CIS Biointernational, 91192 Gif-sur-Yvette Cedex, France). Assays were carried out on supernates of breast cancer homogenates centrifuged at 100 000 × g for 1 h. The optimum cutoff for CD in our study was 40 mmol/g protein, in agreement with our previous findings (7).

ER was assayed by enzyme-linked immunosorbent assays with kits purchased from Abbott Diagnostics,