Development of an enzymatic assay to measure lactate in perchloric acid-precipitated whole blood

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Introduction

• Pyruvate is the end product of glycolysis and the immediate and only precursor of lactate.
• Blood pyruvate concentrations have clinical utility only when measured in conjunction with lactate in the same sample in order to calculate the lactate:pyruvate (L:P) ratio. But pyruvate is measured in whole blood added to perchloric-acid while lactate is measured in whole blood or plasma.
• The objective of this study was to develop a clinical assay for the quantitative determination of lactate in whole blood specimens sent to ARUP for pyruvate testing, so the L:P ratio can be established from the results of these two tests in one specimen.

Method

• Specimen collection and handling:
  - Whole blood anticoagulated with heparin or EDTA was collected from healthy donors and immediately transferred to a 1 mL pre-chilled tube containing 2 mL 8% (w/v) perchloric acid.
  - Samples were mixed well for 30 sec, placed on ice for 10 min, then centrifuged without delay to obtain a protein-free supernatant.
• Lactate measurement:
  Lactate was oxidized to pyruvate by lactate oxidase and the resulting H2O2 produced was oxidized by a chromogen system to a colored chromogen that was detected at 540 nm on a Roche cobas c501 chemistry analyzer.
• Analytical validation:
  Sample processing effects, accuracy, linearity, imprecision, analytical sensitivity, and stability were studied. Reference intervals were established from 116 healthy adults.
• The study was approved by the University of Utah Institutional Review Board.

Results

Figure 1. Alternate methods of sample collection and processing using perchloric acid collection tubes significantly decreased lactate concentrations.

Table 1. Delayed addition of whole blood to perchloric-acid significantly increased lactate concentrations. ANOVA P value = 0.0033

N | Time (min) | Mean lactate (mM) | % increase
--- | --- | --- | ---
5 | 0 | 1.29 | –
5 | 30 | 1.60 | 24.0
5 | 60 | 2.02 | 56.9
5 | 120 | 2.27 | 76.0

Table 2. Precision was determined by measuring lactate in two patient pools in three replicates once each day for 10 days.

Mean concentration | Repeatability | Between-day | Within-laboratory
--- | --- | --- | ---
(mM) | (%) | (%) | (%)
10.89 | 0.6 | 0.5 | 1.1
1.58 | 5.7 | 2.3 | 6.1

Table 3. Recovery was determined by adding lactate to two different concentrations to 4 blood samples prior to adding the sample to perchloric acid.

EDTA | Sample #1 | 105.9 | At 11.89 mM/L, %
| Sample #2 | 105.9 | 111.6
Heparin | Sample #1 | 105.8 | At 6.29 mM/L, %
| Sample #2 | 106.8 | 108.2
| Sample #3 | 103.4 | 112.7

Figure 2. Lactate measured in an acid-precipitated whole blood supernatant was comparable to lactate measured in plasma.

Figure 3. The stability of lactate in a protein-free supernatant was evaluated by four specimens at two concentration levels after storing the samples at three different temperatures over various times. Lactate is stable for 8 hours at ambient, 21 days at 4-8 °C and 30 days at -20 °C.

Conclusions

• Lactate can be quantified in the same protein-free supernatant used for the measurement of pyruvate.
• Alternative methods of sample collection and processing result in falsely decreased concentrations of lactate.
• Delayed addition of whole blood to perchloric acid significantly increased lactate concentrations.
• In a protein-free supernatant, lactate is stable for 8 hours at ambient, 21 days at 4-8 °C and 30 days at -20 °C.
• The lactate reference interval was determined to be 0.31-2.00 mmol/L.
• Determining the L:P ratio from a single sample likely avoids pre-analytical sources of error that can occur when two separate samples are collected and processed for lactate and pyruvate testing.