Clinical Evaluation of an Automated Chemical Inhibition Assay for Lactate Dehydrogenase
soenzyme 1

We evaluated an automated assay for lactate dehydrogenase (LD; EC 1.1.1.27) isoenzymes, supplied by Boehringer Mannheim Diagnostics (BMD) and based on selective chemical inhibition of non-LD-1 isoenzymes by guanidine thiocyanate. Results were compared with the Roche Isomune LD-1 method. The Hitachi 717 analyzer was used to measure enzyme activity for both procedures in 229 serum samples. One hundred specimens were also analyzed by the Helena apid electrophoresis (REP) method. We determined the linear limit of linearity of the BMD method to be about 1200 U of LD-1 per liter. The analytical correlation of BMD (y) with Isomune (x) yielded y = 1.0x + 0.5 U/L, r = 0.997, S_yx = 16.9 (range 10–1397 U/L). The regression equation for BMD vs REP was y = 1.1x + 7.2% (r = 0.800, S_yx = 7.4, range 14–83%). Average values for within-run precision for low (38 U/L), medium (180 U/L), and high (865 U/L) controls were 4.1%, 0.9%, and 0.5%, respectively. Average values for run-to-run precision were 4.1%, 1.7%, and 1.1%, respectively, for these controls (n = 16). We used receiver-operating characteristic curves to determine optimal decision limits. Using an LD-1 cutoff of 40% of total LD, we obtained a clinical sensitivity of 97–100% and a specificity of 95% when blood was collected during the optimum interval, 24–48 h after the onset of chest pain. We conclude that the BMD LD-1 assay is equivalent to the immunochemical and electrophoretic assays for measuring the LD-1 isoenzyme.

Additional Keyphrases: macro LD · chaotropic inhibition ·

1 Department of Pathology and Laboratory Medicine, University of Texas Medical School, Texas Medical Center, P.O. Box 20708, Houston, TX 77225.
2 Clinical Chemistry Laboratory, Hermann Hospital, Houston, TX 77030.
3 Boehringer Mannheim Diagnostics, Indianapolis, IN 46250.
4 Address correspondence to this author.
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Measurement of lactate dehydrogenase (LD, EC 1.1.1.27) isoenzymes is important for differentiating the tissue sources of high total LD activity seen in the serum of patients with various disorders. The five LD isoenzymes are most commonly separated and measured in a clinical laboratory by electrophoresis (1). In actual routine practice, however, most requests for LD fractionation are for the diagnosis of acute myocardial infarction. This has led to the development and evaluation of assays that measure only LD-1, the isoenzyme that predominates in myocardium. Some of these assays include differential inhibition by urea and oxalate (2), ion-exchange chromatography separating LD-1 and LD-2 from LD-3 to LD-5 (3), immunoprecipitation (4), selective inhibition by pyruvate (5) and 1,6-hexanediol (6), and most recently, chaotropic inhibition by sodium perchlorate ("A-Gent LD-1"; Abbott Labs., Abbott Park, IL) (7).

Here, we evaluate as a potential marker for diagnosis of myocardial infarction a chemical inhibition assay for LD-1 isoenzymes that makes use of guanidine thiocyanate to inhibit LD-2 through LD-5. This new procedure is compared with an immunochemical assay and an automated electrophoresis analyzer.

Materials and Methods

Subjects: We analyzed 229 serum samples from 100 patients at Hermann Hospital, Houston, TX. These included 20 normal patients with total LD <200 U/L, multiple samples from 40 patients suspected of myocardial infarction with supranormal total LD and LD-1 activities, and 40 patients with confirmed myocardial infarction. The diagnosis of myocardial infarction was made by attending physicians.

1 Nonstandard abbreviations: LD, lactate dehydrogenase; BMD, Boehringer Mannheim Diagnostics; CK, creatine kinase; and REP, rapid electrophoresis.

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physicians and was based on a positive clinical history, including chest pain, specific electrocardiographic recordings, and serial enzyme values in blood sampled at ~8-h intervals. The enzymes assayed were total creatine kinase (reference range: 12–191 U/L), CK-MB (Stratus CK-MB; Baxter Healthcare Corp., Dade Div., Miami FL, ref. range: <9 μg/mL, relative index <1.5), total lactate dehydrogenase (ref. range: 83–200 U/L), and LD-1 (ref. range: <40% of total LD). Results of the LD-1 assay by the chemical inhibition method were not made known to the attending physicians and therefore were not used in the diagnosis. In many cases, cardiac catheterization was performed and the diagnosis was confirmed by angioscopy. We also studied two samples containing macro LD. The electrophoretic pattern of one of these is very similar to that reported by Sudo et al. (8).

Procedures: We analyzed the serum samples by electrophoresis, using the Rapid Electrophoresis System (REP; Helena Laboratory, Beaumont, TX), and the immunoprecipitation assay (Isomune LD; Roche Diagnostics, Nutley, NJ). Results from these were compared with those obtained from the chemical inhibition method [LD-1; Boehringer Mannheim Diagnostics (BMD), Indianapolis, IN]. Samples are preincubated with guanidine thiocyanate (190 mmol/L) and lactate (92 mmol/L) for 5 min before analysis for residual LD activity. Both the immunoprecipitation and chemical inhibition methods as well as total LD were measured in the Hitachi 717 analyzer (BMD). Figure 1 illustrates the effectiveness of the chemical inhibition when a sample is electrophoresed before and after treatment with guanidine thiocyanate.

Stability: We prepared fresh specimens with low total LD (<200 U/L), medium total LD (>200 U/L, LD-1 <40% of total), and high total LD (>200 U/L, LD-1 >40%). A specimen from each group was divided into 24 aliquots. Eight aliquots of each sample were stored at room temperature, eight at 5–6 °C, and eight at −20 °C. One aliquot from each storage condition was analyzed each day for seven days and on the 15th day. The analyses were performed for both total LD and LD-1.

Precision: Control specimens with low (38 U/L), medium (180 U/L), and high (865 U/L) values for LD-1 were prepared. Each specimen was divided into eight aliquots and stored at 5–6 °C. One aliquot from each category was analyzed each day for the next seven days. Each day, one aliquot from each group was divided into six sample cups (n = 18) and analyzed twice each day for LD-1.

Linearity: A control specimen with 1126 U of LD-1 activity per liter was serially diluted, up to 10-fold, with isotonic saline. The same specimen was similarly diluted with a serum specimen that had an LD-1 activity of 10 U/L. We analyzed these diluted samples and the two diluents for LD-1 activity, using the BMD LD-1 assay.

Results

Stability: Because there is disagreement in the literature over the stability of LD isozymes (9), we performed this study to determine how the BMD LD-1 assay would perform with specimens stored at different temperatures. In addition, we wanted to determine which storage condition was best suited for the precision study and to what extent storage would affect the data.

The results indicate that, with use of the BMD LD-1 assay, there was no loss of activity in specimens stored at room temperature, at 5–6 °C, or frozen for as long as a week. Similar results were obtained for total LD. The stability of total LD at −20 °C is somewhat unexpected because LD-4 and LD-5 from tissue extracts have been shown to be unstable when frozen (9).

Precision: Statistical analyses of the data from the precision study show good precision by the chemical inhibition method. The average within-run precisions (CVs) for the pooled low, medium, and high sera were 4.1%, 1.0%, and 0.5%, respectively (16 trials of six each). Their average run-to-run precisions were respectively 4.1%, 1.7%, and 1.1% (n = 16); day-to-day precisions were 4.0%, 2.0%, and 1.0% (12 trials for eight days).

Linearity: Figure 2 illustrates the results of the linearity study when saline was used as the diluent. We computed linear regression, using the lowest six dilutions. The predicted values for the higher dilutions were extrapolated from this curve. The results show that, within a 5% tolerance, the BMD LD-1 assay is linear up to at least 1200 U/L. Similar results were obtained when serum containing 10 U of LD-1 per liter was used as the diluent.

Analytical correlation: Results of the chemical inhibition method were compared with REP for determining the LD-1 percentage of total LD. Although values of the electrophoretic procedure were lower, there was a good analytical correlation between the two methods over the LD-1 range of 14% to 83% of total LD. Figure 3 illustrates their linear correlation, the equation for which is y = 1.1x + 7.2 (r = 0.800, S_eyx = 7.4, n = 100).

The comparison between the BMD LD-1 assay and Isomune yielded a better analytical correlation than did the comparison with electrophoresis. The choice of U/L instead of LD-1 percentage of total LD was necessary to extend the range of the linear graph. The linear correlation graph yielded the equation y = 1.6x + 0.5 U/L (r = 0.987, S_eyx = 16.9, n = 220) for the range 20–1397 U/L (Figure 4).

Because 40% of total LD is the LD-1 cutoff value specified...
in the Roche Isomune inhibition test for abnormal results, an equivalent value was established for the BMD LD-1 assay. Analytical correlation between the two procedures was performed for LD-1 in a range of 0% to 40% of total LD. The linear-correlation curve yielded $y = 0.818x + 6.08 (r = 0.820, S_x = 3.3, n = 103)$. From this equation, the 40% value by Roche Isomune (x) is equal to 39% (y) of the predicted value with the new method. (The equivalent correlation equation when these data were plotted in U/L was $y = 0.99x - 0.57 U/L, S_x = 9.0, r = 0.960$.)

Clinical sensitivity and specificity: Receiver-operating characteristic curves were used for determining clinical sensitivity and specificity (Figure 5). Sensitivity is the percentage of true positives divided by the sum of true positives and false negatives, and specificity is the percentage of true negatives divided by the sum of true negatives and false positives. From the calculated data used in these curves, we selected an LD-1 cutoff of 40% of total LD. We obtained values of 80% and 95%, respectively (Figure 5, top), for all the samples (n = 114) collected from myocardial infarct patients within 48 h after onset of chest pain for both the BMD LD-1 and the Roche Isomune assays. Table 1 lists the clinical sensitivity and specificity for these data, divided into time intervals after the reported onset of myocardial infarction for the BMD LD-1 assay. The increase in serum LD-1 concentration starts 8–12 h after the onset of chest pain, reaching the maximum in about 24–48 h. This explains the increasing sensitivity of the assay with time as shown in Table 1.

Macro LD samples: We also evaluated the performance of the LD-1 assay for the two samples containing macro LD isoenzymes. Figure 6 shows the electrophoretic LD isoenzyme pattern of one sample. When this was electrophoresed after incubation with the inhibitor, all bands except LD-1 were inhibited. Quantitative results by the chemical inhibition assay also compared well with electrophoresis and immunoprecipitation for sample 1 (LD-1, % of total LD = 28% vs 30% and 33%, respectively) and sample 2 (5% vs 5% and 7%, respectively). These findings suggest that macro LD does not produce falsely positive results in the BMD LD-1 assay.

Discussion

Retrospective diagnosis of acute myocardial infarction involves confirmation of a triad of findings, including a positive clinical history, specific electrocardiographic changes, and increases in serum enzymes measured serially. For the last, most cardiologists place heavy reliance on measurement of total creatine kinase and its CK-MB isoenzyme. As a result, there are extensive commercial

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**Table 1. Clinical Sensitivity and Specificity of the BMD LD-1 Assay**

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
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<tbody>
<tr>
<td>6-12</td>
<td>39</td>
<td>95</td>
</tr>
<tr>
<td>12-24</td>
<td>86</td>
<td>95</td>
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<td>24-36</td>
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<td>36-48</td>
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<td>95</td>
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<tr>
<td>0-48</td>
<td>80</td>
<td>95</td>
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* Cutoff value of LD-1/total ≥ 40%.
efforts aimed at developing more sensitive and specific assays for CK-MB (10).

Largely overlooked, however, is the clinical utility of the LD-1 isoenzyme. There may be several reasons why requests for LD isoenzymes do not parallel those for CK-MB. First, the clinical efficiency for diagnosis of LD-1 for myocardial infarction is not as high as CK-MB when LD isoenzymes are measured by electrophoresis and a "flipped" ratio of LD1/LD2 > 1 is used as the cutoff. Therefore, the perception is that this inefficiency has a pathophysiological basis. In reality, Leung and Henderson (11) showed that greater sensitivity and specificity can be obtained when a more appropriate ratio of 0.76 is used as the decision limit. Many laboratories nevertheless continue to use the 1.0 cutoff because it is more convenient to use to match LD-1 and LD-2 peak heights.

A second reason is the perception that LD-1 isoenzymes are useful only after concentrations of CK-MB have peaked, some 18-24 h after infarct. Contrary to this, Bruns et al. (12) showed that measurement of LD-1 had a sensitivity of 90% at 12 h after admission. Adam et al. (12) concluded that LD-1 measurements are actually superior to CK-MB when the latter is measured by immuno inhibitory, particularly for those infarct patients who have multiple problems.

Our results confirm the notion that LD-1 isoenzyme measurements should be used more widely for diagnosis of myocardial infarction. Although we did not specifically compare LD-1 with CK-MB in this study, historical data show that the efficiency of LD-1 is at least equal to that of CK-MB, beginning 12 h after myocardial infarction onset. With the development of a homogeneous assay for highly automated chemistry analyzers, LD-1 results are more easily available and more cost effective than are results for CK-MB. The new procedure is convenient to use and can produce results within 10 min. We recommend the use of a relative value of LD-1/total LD > 40% as a decision limit for diagnosis of myocardial infarction. This is particularly useful when total serum LD is increased owing to non-myocardial sources, which will increase the absolute value of LD-1 in the serum. However, an absolute cutoff value in U/L is useful when the total LD is only marginally increased.

References