Optimization and Evaluation of Cardiac Enzymes and Isoenzymes Measured on a Random Access Analyzer

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ABSTRACT

Four serum enzymes and isoenzymes used in the diagnosis of acute myocardial infarction (AMI), lactate dehydrogenase LD and LD-1, creatine kinase (CK), and CK-MB have been adapted to the Technicon RA-1000 automated clinical chemistry analyzer. Analytical parameters have been adjusted to provide clinically acceptable precision for all four assays. Correlations with centrifugal analyzer procedures gave correlation coefficients ranging from 0.998 to 0.999. A limited clinical study of the CK-MB assay indicated that a discriminant value of 13 U per L could separate AMI from non-AMI patients.

Introduction

Measurements of the MB isoenzyme of creatine kinase (CK-MB) and lactate dehydrogenase isoenzyme 1 (LD-1) in serum provide reliable information for the diagnosis of acute myocardial infarction (AMI). The development of immunoassisted methods for these two isoenzymes for the centrifugal analyzer has been reported by the present authors.2, 3, 4 These reports included clinical as well as methodological evaluations.

The recent introduction of random access analyzers into the clinical laboratory has simplified work-flow problems associated with the older batch analyzers. These random access analyzers can be operated to perform measurements of a variety of equilibrium and kinetic reactions and are well suited to enzyme assays. However, the clinical value of CK-MB and LD-1 depends on good analytical precision at low activity values. The present study was initiated to adapt lactate dehydrogenase LD, creatine kinase (CK), and the immunoassisted LD-1 and CK-MB assays to one of the more popular random access analyzers* and to attempt to make modifications to achieve adequate precision for diagnostic purposes.

* Technicon RA-1000, Tarrytown, NY 10591.
Materials and Methods

The immunochemical and enzyme reagents and the immunochemical methods for initial sample treatment were as described previously by the present authors. Briefly, the reagent kit for the immunochemical separation of LD-1 from the other four isoenzymes of LD was obtained from a commercial source. Total LD activity was measured using the pyruvate to lactate reaction with commercially available reagents.

The reagents for the immunochemical separation and selective inhibition of the isoenzymes of CK were obtained from a commercial source. The activities of CK-MB and of total CK were measured using an optimized assay reagent (BMC CK NAC). This enzyme reagent was modified by the addition of 5M magnesium acetate to overcome the EDTA present in the immunochemical reagents.

The random access clinical analyzer (Technicon RA-1000)* was operated according to the manufacturer's instructions except for the modification of the sample syringe used in the CK-MB activity measurements. The modification of the syringe was simply a substitution of a 100 μl for the original 50 μl syringe. This exchange of syringes can be accomplished in approximately 30 seconds. The Technicon RA-1000 is capable of using seven or nine absorbance readings taken 15 or 30 seconds apart. Automatic regression analysis of these absorbances is used to compute enzyme activity. For the CK-MB measurements the computation program was forced to use nine absorbance readings with a 30 second interval. In addition an absorbance window was selected to reject analyses demonstrating spectrophotometric imprecision. This window was ±1.2 milliabsorbance around the regression line. Any absorbance reading outside of this window caused the analysis to be rejected.

Instrument settings for all of the enzyme measurements are listed in table I. For LD-1 and CK-MB, the volumes listed are those of the solution following the immunochemical separation and inhibition, and not of original sample. All measurements were carried out at 37°C and 340 nm.

Comparison Procedures

Correlation studies of CK, LD, and LD-1 were carried out with the RA-1000 and a centrifugal analyzer. The centrifugal analyzer methods were described in our earlier studies. All of the activity measurements on the centrifugal analyzer were made at 30°C.

Electrophoresis is the technique used by many laboratories for semi-quantitation of CK-MB. One of our previous studies compared CK-MB quantitation by centrifugal analyzer with electrophoretic estimation by fluorometric staining using the Corning ACL system. This comparison with electrophoresis was repeated in the current study using the optimized RA-1000 random access analyzer method. Fifty-eight patient samples were selected for this comparison from among specimens submitted to the laboratory for CK-MB quantitation. Since we were most concerned with the performance of the assay at low CK-MB values, only samples with CK-MB activities less than 38 U per L were chosen. Patient records were reviewed to determine clinical diagnoses. Electrophoresis results were graded as “negative”, “trace”, or “positive”.

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* Roche Diagnostics, Nutley, NJ 07110.
† BMC UV System LDH-P; Bio-Dynamics bmc, Indianapolis, IN 46250.
‡ Hamilton Co., Reno, NV 89520.
§ Hamilton Co., Reno, NV 89520.
¶ Corning Medical, Medfield, MA 02052.

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CHOICE OF ENZYME REAGENTS

The pyruvate to lactate procedure for LD assays was chosen as the routine assay in the authors' laboratory since it follows the natural equilibrium with a rate 2.5 time that of the reverse reaction. The CK activity was determined using reagents recommended by Szasz with the important additions of 10.2 μM diadenosine-5'-pentaphosphate to inhibit adenylate kinase and 2.0 mM ethylene diamine tetracetic acid (EDTA) as cation chelator which has been shown to increase CK activity. The CK assay chosen in the present study was a two reagent system in which the CK in the sample is activated with sulfhydryl during the first delay (180 s) and the reaction then is triggered with creatine phosphate. Preincubation of sample with sulfhydryl activator has been reported to produce more complete reactivation of CK in some samples. An additional advantage of this approach over a single reagent system is that substrate is not utilized during the time of enzyme activation and thus the linear range of the assay is increased. The present method is linear to 2300 U per L whereas with a single reagent method the corresponding linearity is approximately 1150 U/L.

As mentioned earlier, both LD and CK assays are available in commercial kits. There are several immunoinhibition kits available for CK-MB. However, the only one which is free from interferences from CK-BB, adenylate kinase and atypical CK, is that chosen for the present investigation. Reagents from the same company were used for the immunoprecipitation assay for LD-1 since, to the author's knowledge, these are the only ones available commercially. The principles of these methods have been described clearly by their originators.

Results and Discussion

Sensitivity and Precision

The sensitivity and precision of the analyses are closely linked. One factor affecting the sensitivity of the Technicon RA-1000 is that the optical light path is 7 mm rather than the usual 10 mm. Thus, these absorbance readings are less than with a more conventional optical system. Another limiting feature of the RA-1000 is that an unmodified instrument allows a maximum sample volume of only 30 μl. Respecting these limitations, assays for LD, LD-1, CK, and CK-MB were developed and gave sensitivities shown in table II. These sensitivities are expressed in absorbance change per minute per unit and absorbance change per minute at the decision value. The parameters for all assays were as shown in table I except for CK-MB where an

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<thead>
<tr>
<th>Table I: Parameters for RA-1000 Enzyme Measurements</th>
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<tr>
<td>Sample vol. (μl)</td>
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<tr>
<td>12</td>
</tr>
<tr>
<td>360</td>
</tr>
<tr>
<td>60</td>
</tr>
<tr>
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<tr>
<td>37</td>
</tr>
<tr>
<td>340</td>
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<table>
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<tr>
<th>Table II: Sensitivities of the Enzyme Assays</th>
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<tbody>
<tr>
<td>LD</td>
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<tr>
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<tr>
<td>0.12</td>
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<tr>
<td>0.084</td>
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<tr>
<td>0.15</td>
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<tr>
<td>0.34</td>
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Decision values are upper limits of the reference range and are given in parentheses.

* Roche Diagnostics, Inc.
additional non-optimized assay with a sample volume of 30 µl is listed. The obvious improvement in sensitivity can be seen using the 60 µl sample size. Also, it should be noted that the combined volume of the two reagents for CK-MB is less than for total CK and represent approximately the minimal volume acceptable by the instrument.

The precision of the four total enzyme and isoenzyme assays using optimal conditions is shown in table III. These data represent analyses carried out over a 24-day period using the two commercial controls. The sensitivities and precision of the LD, LD-1, and CK assays were satisfactory using parameters on the RA-1000 similar to those used for our original clinical studies on the centrifugal analyzer. The obvious differences in the two instruments such as optical path length (7 mm vs. 10 mm) and the necessity of using a sample diluent on the centrifugal analyzer, did not affect the overall performance of the assay. However, there were major problems with the CK-MB assay especially at the decision level, which in our laboratory is 13 U per L. Our original assay using 30 µl of supernatant for the immunoprecipitation reaction provided precision of ± 20 percent at this decision level. This level of imprecision made the assay of limited value for the diagnosis of acute myocardial infarction and it became essential to provide a means of improving the sensitivity and, therefore, the precision of the procedure. The major change made to improve the precision of this assay was to substitute a 100 µl sample syringe for the original 50 µl syringe which allowed us to increase the maximum sample volume from 30 µl to 60 µl. The total sample and reagent volume was 345 µl which is only slightly above the minimal amount which can be safely used in the RA-1000 cuvettes. Also, the authors became aware of the fact that the immunoinhibition reagents for CK-MB provided by the manufacturer* contained EDTA. The reagents used for the total CK assay† also contained EDTA and magnesium supplementation for activation of the CK. We found it necessary to increase the magnesium concentration in the substrate to overcome the effects of the additional EDTA in this immunoinhibition reagent.

**Correlation Studies**

The new procedures for CK, LD, and LD-1 were correlated with similar meth-

* Roche Diagnostics, Nutley, NJ 07110.
† BMC UV Systems, Indianapolis, IN 46250.
ods which we had adapted for use on a centrifugal analyzer and which had been in routine use in our clinical laboratory for several years, and the results are shown in table IV. As expected these correlation studies showed higher values on the Technicon RA-1000 which was operated at 37°C rather than 30°C used on the centrifugal analyzer. The non-zero Y intercepts can be explained by the differential effect of temperature on reaction rate which has been reported for LD$^6$ and also aspartate aminotransferase.$^{10,11}$

The results of the comparison of CK-MB determinations by electrophoresis and Technicon RA-1000 are illustrated in figure 1. This plot is similar to that seen in our previous comparison of CK-MB data from electrophoresis and centrifugal analyzer methods.$^2$ In the current study, samples which contained no CK-MB activity detectable by electrophoresis had quantitative CK-MB activities less than or equal to 10 U per L. Samples “positive” for CK-MB by electrophoresis had CK-MB activities exceeding 10 U per L with one exception. The exception involved a patient who had undergone coronary artery bypass grafting the previous day. Samples showing “trace” CK-MB activity by electrophoresis had intermediate quantitative values.

Twelve of the 58 patient samples were from three patients with the clinical diagnosis of acute myocardial infarction. Peak CL-MB values for each patient exceeded 13 U per L. These CK-MB values were higher than those reported in our earlier clinical study$^2$ since the present assay was carried out at 37°C rather than the earlier 30°C.

**Conclusion**

The excellent analytical performance of the LD, LD-1, and CK assays coupled with the correlation data provided us with confidence that these assays could provide valid results when in routine clinical use. We made this judgment based upon several years of experience using very similar assays on the centrifugal analyzer. However, the borderline sensitivity of the CK-MB assay at a decision level of 13 U per L indicated that a limited clinical study and comparison with electrophoresis were required. This study was carried out on patients and the data are presented in figure 1. From these data it can be concluded that the assay can provide excellent discrimination between AMI and non-AMI patients.

**References**