strates that cardiac decompensation can be avoided in MCD-deficient patients. Finally, by analogy with fatty acid oxidation disorders such as medium-chain acyl-CoA dehydrogenase deficiency (13), one may conclude that knowledge of the diagnosis will improve outcome because early intervention is then possible in catabolic episodes that otherwise may lead to metabolic derangement. It therefore seems reasonable to suppose that early diagnosis and treatment can reduce the morbidity and mortality associated with MCD deficiency and to include MCD deficiency in neonatal metabolic screening programs. In this study, the malonylcarnitine concentration in the neonatal screening test card was increased in only one of the two patients investigated, but this was a problem of stability after sample storage for several years.

We conclude that (a) malonylcarnitine is detectable by ESI-MS/MS, (b) malonylcarnitine is increased in the blood of patients with malonic aciduria, and (c) ESI-MS/MS-based neonatal screening programs should be able to detect patients with MCD deficiency, a treatable metabolic disorder, before the development of symptoms.

References


IFCC Method for Lactate Dehydrogenase Measurement in Heparin Plasma Is Unreliable, Andries J. Bakker, Bidiaiperkash Mirchi, Johannes T. Dijkstra, Freerk Reitsma, Heye Syperda, and Appie Zijlstra (Department of Clinical Chemistry, Klinisch Chemisch Laboratorium, PO Box 850, 8901 BR Leeuwarden, The Netherlands; * author for correspondence: fax 31-582882227, e-mail a.j.bakker@kcl.znb.nl)

Various societies for clinical chemistry have proposed recommendations for the measurement of the activity of lactate dehydrogenase (LDH; l-lactate:NAD+ oxidoreductase; EC 1.1.1.27) (1–5). Depending on the pH of the buffer, the activity of LDH can be measured by the increase as well as the decrease in NADH. The optimum pH for the pyruvate-to-lactate conversion is 7.4–7.8. The German (2) and French (5) Societies for Clinical Chemistry recommend this reaction. The IFCC (4) recommends the lactate-to-pyruvate conversion, for which the optimum pH is 8.8–9.8.

In our laboratory, we have measured LDH according to the recommendations of the French society (SFBC) for years. This measurement was routinely performed with a Modular analyzer (Roche GmbH). When this SFBC method for LDH measurement needed to be replaced because our supplier stopped producing the necessary reagents, we decided to introduce the method based on the recommendations of the IFCC. Because we did not want to change our reference values (<450 U/L), we introduced a conversion factor to make the results obtained with the IFCC method directly comparable to the results obtained with the SFBC method.

When we compared both methods using patient samples for which LDH was requested, we found numerous outliers, which forced us to do some additional investigations. To determine which method was responsible for the outliers, we began measuring duplicates for LDH with both methods. For these measurements we used lithium-heparin-plasma samples collected in plastic tubes with plasma separator (product no. 367994; Becton Dickinson). All measurements took place in the primary tubes, i.e., LDH measurements were performed directly in the lithium-heparin plasma after plasma was separated from the blood cells by centrifugation (1800 g for 5 min), but without pipetting the plasma to secondary tubes. In these experiments, we found a rather high frequency of duplicate errors for LDH measurements with the IFCC method (27 of 152; 17.8%), whereas we found no such excessive frequency of duplicate errors with the SFBC method (2 of 140; 1.4%; Fig. 1A). Duplicate errors were defined as those differences that exceeded the 95% confidence limits for the difference between duplicates.

To estimate the 95% confidence interval for the difference between duplicates, it is important to know the standard deviation of the assay (SDa) used. In our hands the CV for the LDH measurements according to the IFCC as well as the SFBC was ±2%; for the IFCC method, control 1 gave a mean of 344 U/L with a CV of 1.3% (n = 198), and control 2 gave a mean of 813 U/L with a CV of 1.1% (n = 198); for the SFBC method, control 1 gave a
mean of 343 U/L with a CV = 1.8% (n = 400), and control 2 gave a mean of 858 U/L with a CV of 2.0% (n = 400). When the difference between duplicate measurements was considered, the variation of the resulting difference was: 

\[
SD_r = SD_a \times \sqrt{2}
\]

Therefore, the 95% confidence interval for the differences between duplicates should be

\[
0 \pm 2 \times SD_r.
\]

Because the low centrifugation time and speed (5 min at 1800\(g\)) of our Modular system might be responsible for insufficient elimination of cells from the plasma, we first increased centrifugation time and speed to 8 min at 1800\(g\). With these conditions, the frequency of duplicate errors was 21\% (19 of 91). We then increased the speed and time by centrifuging off-line (MSE Mistral 3000i; Sanyo Gallenkamp) for 10 min at 2500\(g\). The frequency of duplicate errors under these conditions was 18\% (14 of 79). We therefore concluded that the high frequency of duplicate errors probably was not related to inappropriate sample preparation causing insufficient elimination of cells from the plasma.

To eliminate the possibility that the occurrence of this high frequency of duplicate errors was caused by interference from another test, we repeated the experiment in a “clean” Modular analyzer, i.e., the analyzer was rinsed extensively and duplicate measurements of LDH were performed without the concurrent measurement of other analytes. The frequency of duplicate errors was 28.7\% (60 of 209; Fig. 1B). To determine whether it was the analyzer or the method that caused the problem, we repeated the experiment with duplicate LDH measurements with a Hitachi 911 analyzer and a Hitachi 717 analyzer (Roche GmbH). Both analyzers showed the same problem. The frequency of duplicate errors was 34\% (27 of 79) for the Hitachi 911 and 18\% (5 of 27) for the Hitachi 717. To investigate whether it was the IFCC method for LDH or the type of sample that caused the duplicate errors, we performed duplicate measurements in primary tubes with serum (with serum separator; product no. 367955; Becton Dickinson) because serum is the preferred specimen according to the various recommendations. The frequency of duplicate errors with serum was low (3 of 114; 2.6\%). In another experiment, we transferred the plasma sample to a secondary tube. Because such a transfer caused efficient mixing and therefore homogenized the sample, the frequency of duplicate errors was almost absent in this experiment (1 of 94; 1.1\%).

As recommended by our analyzer supplier, we used plastic tubes for routine analysis in our modular analyzer. In an experiment with glass tubes with lithium-heparin and plasma separator (product no. 367793; Becton Dickinson), we found approximately the same high frequency of duplicate errors (29 of 148; 19.6\%) compared with plastic tubes. Thus, the effect of tube material was excluded. In addition, we studied the effect of plasma separator and could not attribute any effects to the application of plasma separator: the frequency of duplicate errors was 19\% (8 of 42) for heparin plasma with plasma separator and 35\% (18 of 52) for heparin plasma without plasma separator.

According to the recommendations of the various societies, serum is considered the preferred specimen because plasma may be contaminated with platelets, which contain high concentrations of LDH. The advantages of heparin plasma, including (a) that it is available for analysis sooner than serum, which needs at least 30 min of clotting; (b) that hemolysis is less extensive in plasma than in serum; and (c) that the absence of clotting in plasma minimizes the release of LDH from platelets, makes plasma the preferred specimen for rapid routine analysis. Furthermore, the development of analyzers that include sample preparation units supports the use of heparin-
plasma samples. In the past, we did not encounter duplicate problems with heparin-plasma samples when we used the SFBC-recommended LDH method, which again was demonstrated in a separate experiment in this study. Thus, there could be reasonable doubt whether the duplicate errors that occurred with the IFCC method were caused by contamination of the plasma with platelets or erythrocytes because then the SFBC method also would be expected to have a high frequency of duplicate errors. The fact that optimization of the centrifugation procedure did not significantly alter the frequency of duplicate errors supported this view. However, based on the differences in buffer composition (pH and NaCl content), one might also suppose that when contamination by platelets or erythrocytes occurs, their integrity may be better preserved under the conditions of the SFBC method than under the conditions of the IFCC method. In this case, the presence of cells might play a role in the high frequency of duplicate errors. Nevertheless, this high frequency of duplicate errors for the IFCC method from Roche for their modular analyzer is unacceptable for routine analysis and does not provide reliable results for LDH in heparin-plasma samples.

References

Capillary Electrophoresis Method to Measure p-Ammonihippuric Acid in Urine and Plasma for the Assessment of Renal Plasma Flow, Paula M. Ladwig, Jan H. Bergert, and Timothy S. Larson (Divisions of 1 Clinical Biochemistry and Immunology and 2 Nephrology, Mayo Clinic and Foundation, Rochester, MN 55905; * address correspondence to this author at: Mayo Clinic, 200 First Street SW, Rochester, MN 55905; fax 507-284-5470, e-mail larson.timothy@mayo.edu)

p-Ammonihippuric acid (PAH), a derivative of aminobenzoic acid, is almost completely extracted from the blood after a single passage by the kidney through a combination of glomerular filtration and proximal tubular secretion. On the basis of these properties, the renal clearance of intravenously administered PAH has been used as a measure of renal plasma flow (RPF) (1). PAH remains the “gold standard” for the noninvasive measurement of RPF in patients and study participants and may be useful for assessing the effect of disease states or pharmacologic agents on renal function.

The standard method for PAH measurement is a colorimetric assay of a diazotation reaction that is labor-intensive (2, 3). Because of the complexities of the standard colorimetric PAH assay, its measurement has often been confined to research or used only in specialized laboratories. Measurement of PAH in urine and plasma by HPLC has been described (4–7), but it requires relatively large sample volumes and time-consuming extraction procedures. We recently found that capillary electrophoresis (CE) is an efficient, inexpensive, and reliable method for measurement of the nonradiolabeled iothalamate in urine and plasma samples for the assessment of glomerular filtration rate (8, 9). Analysis by CE is analytically faster than standard HPLC or colorimetric assays, requires less reagent preparation and smaller sample size, minimizes drug interferences, and improves test turnaround time. With the development of a method for PAH on CE, assays for both RPF and glomerular filtration rate could also be obtained with a single methodology. This report describes a new quantitative CE assay for PAH in urine and plasma and compares it with the standard colorimetric assay.

PAH was measured by CE on a Beckman Pace instrument using Beckman System Gold software, 50 mmol/L borate buffer (pH 10.2), ultraviolet detection at 280 nm, and a fused-silica capillary (length, 37 cm) from Polymicro Technologies Inc. The temperature was kept at 21 °C and the voltage at ~25 kV; injection was induced by positive pressure. Separation was positive to negative, and the retention time for PAH was ~1.90 min.

Initially the capillary was prepared by perfusing for 20 min with 0.10 mol/L NaOH, 20 min with water, and 20 min with 50 mmol/L borate (pH 10.20). The daily coil preparation consisted of a shortened 2-min program. Capillaries were cleaned between sample injections, which eliminated any detectable carryover. Between each sample, a cycle of base, water, and buffer was run through the capillary for ~30 s each. A calibrator was run to check the retention time of the PAH peak. Voltage was adjusted to attain a retention time of 1.80–2.00 min for the PAH peak.

The calibration curve was prepared from six working PAH calibrators: 50, 20, 10, 5, 3, and 1 mg/L PAH (Sigma Chemical Company). From the calibration curve (R² = 0.9997), the PAH peak areas were used for calculating the PAH concentrations in patient samples. A urine control (15–25 mg/L, diluted values) and a plasma control (20–25 mg/L, undiluted values) were prepared from patient samples, and aliquots were stored at ~70 °C.

Precision was assessed for both within-day and between-day analyses. For the within-day precision, two samples at different concentration ranges were assayed 10 times in 1 day for both urine and plasma. The between-day precision was assessed using samples and controls until 20 points were obtained for both. The mean CV was