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-Aminohippuric Acid in Urine and Plasma for the Assessment of Renal Plasma Flow, Paula M. Ladwig,1 Jan H. Bergert,1 and Timothy S. Larson1,2 (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-Aminohippuric 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 iohalamate 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 System Gold 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 caliberator 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
then used. For plasma, the within-day CV was 3.0% and the between-day CV was 4.9%. For urine, the mean within-day CV was 2.1% and the mean between-day CV was 4.7%. The CV for the controls was 7.1%.

To evaluate linearity, we prepared 20-, 40-, 60-, 80-, and 100-fold dilutions of a urine sample and 1-, 0.6-, 0.5-, 0.4-, and 0.3-fold dilutions of a plasma sample. Each dilution was assayed four times and averaged. The averaged results were plotted against the expected results, and regression was calculated. The results expected (x) did not differ significantly from those obtained (y) for urine or plasma. The linear regression equations for this analysis were:

\[ y = 0.96x + 1.35 \text{ mg/L} \quad (R^2 = 0.9977) \]  
\[ y = 0.88x + 2.01 \mu g/L \quad (R^2 = 0.9983) \]  

Recovery of PAH was assessed in both urine and plasma. To each of five urine samples (1 mL) we added 0.1 mL of 28.67 mg/L PAH. Each sample was filtered, diluted 1:30 with 5 mmol/L borate buffer (15 µL of sample in 435 µL of buffer), and assayed twice. The results were averaged. To plasma we added 10, 20, 40, and 100 mg/L PAH. Each sample was deproteinized, diluted 1:10 with 5 mmol/L borate buffer (50 µL of sample in 450 µL of buffer), assayed four times, and averaged. The mean (SD) recoveries were 102.6% (4.0%) for urine and 103.9% (4.4%) for plasma.

Plasma and urine pooled controls were stable for 1 month at 4 °C and 3 months frozen at −20 °C.

Heparinized blood samples were centrifuged for 7 min at 800 g. We then diluted 100 µL of the plasma with 400 µL of 5.0 mmol/L borate and deproteinized 100 µL of the diluted plasma with Amicon Microcon-30 ultrafilters (30 000 molecular weight cutoff) centrifuged 10 min at 11 000 g. We deproteinized 300 µL of urine with Amicon Microcon-30 ultrafilters centrifuged 10 min at 11 000 g. The deproteinized urine was then diluted 1:100 with 5.0 mmol/L borate (10 µL of urine in 990 µL of buffer). Split plasma and urine samples were assayed by both CE and colorimetric methods, and the RPF was calculated as outlined below.

The colorimetric assay for PAH is described in detail elsewhere (3). Briefly, PAH is diazotized by sodium nitrate, and the excess sodium nitrate is neutralized by ammonium sulfamate. The diazotized PAH combines with N-(1-naphthyl)ethylenediamine dihydrochloride (NEDD) to form a purple-colored complex, which is quantitated on a Technicon Auto Analyzer at 550 nm. Calibrators and controls are analyzed in the same way.

Patients (n = 112) referred by their physicians for measurement of RPF participated in a method comparison study of CE vs the colorimetric assays of PAH for RPF measurement. A priming solution was prepared containing 0.05 mL of 200 g/L PAH per kg of body weight diluted to a final volume of 20 mL with diluent fluid. The sustaining solution was prepared by multiplying the
patient’s estimated function by 15 to give the mL of 200 g/L PAH diluted to a final volume of 200 mL with intravenous diluent. Patients were kept fasting for 4 h before the test (patients with diabetes mellitus for 2 h). In the hour preceding the test, patients were instructed to drink four glasses of water. Patients were also asked not to void within 45 min of their appointment time.

At the start of the test, patients were asked to void a pretest urine sample \( (U_0) \), and an initial blood sample was drawn \( (P_0) \), followed by intravenous administration of the primary solution over a 2-min period and initiation of the sustaining solution at 1 mL/min. After the priming dose was completed, a hydrating solution of 25 g/L dextrose was attached with a flow rate of 2–3 mL/min. After a 45-min equilibration period, patients were asked to void \( (U_1) \). A blood sample was also drawn \( (P_1) \) within 5 min of the urine collection from the arm opposite the one used to administer intravenous solutions. After 30 min, patients were asked to empty their bladders again \( (U_2) \), and time and volume were recorded. Another blood sample was immediately taken \( (P_2) \). Bladder voiding and blood draws were repeated every 30 min until samples \( U_3 \) and \( P_4 \) were obtained. Completeness of bladder emptying for all urine collections was monitored with a portable ultrasound bladder scanning device (Diagnostic Ultrasound). The urine volume \( (mL) \) was divided by the collection time \( (min) \) to give the flow \( (mL/min) \). The RPF \( (mL/min) \) for period 1 equaled \( \frac{\text{flow} \text{(mL/min)}}{\text{average of the concentration} \text{(mg/L)}} \times \frac{\text{body surface area} = \text{height} (cm)^{0.725} \times \text{weight} (kg)^{0.425}}{11002} \times \frac{0.007184}{11003} \).

The results of the method comparison for the split urine and plasma samples measured by CE and colorimetric methods are shown in Fig. 1, A and B. The RPF values obtained by CE and colorimetric methods for all study participants are compared in Fig. 1C. The overall mean RPF for CE was 322 mL·min\(^{-1}\)·m\(^{-2}\) and for colorimetric was 322 mL·min\(^{-1}\)·m\(^{-2}\) with an \( R^2 \) of 0.979.

In conclusion, measurement of urine and plasma PAH by CE is an accurate alternative to the colorimetric assay for assessment of RPF. This new CE method reduces drug interferences, analysis time, and required volumes of reagents and blood. The method comparison results, recoveries, and assay variation are all acceptable for this new methodology. With the CE method, there is less reactant preparation than with the colorimetric assay, which required preparation of many calibrators. Finally, computer-controlled instrumentation for the CE assay also allows for automation and eliminates time-consuming calculations.

References

Population Distributions of C-reactive Protein in Apparently Healthy Men and Women in the United States: Implication for Clinical Interpretation, Nader Rifai, and Paul M. Ridker (1) Department of Laboratory Medicine, Children’s Hospital, Boston, MA 02115; (2) Center of Cardiovascular Disease Prevention and (3) Division of Cardiovascular Disease, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA 02115; * address correspondence to this author at: Children’s Hospital, Department of Laboratory Medicine, 300 Longwood Ave., Boston, MA 02115; fax 617-713-4347, e-mail nader.rifai@tch.harvard.edu

Measurement of the acute-phase reactant C-reactive protein (CRP) has been used historically in the diagnosis and monitoring of active infection or inflammation. Recent prospective epidemiologic studies have demonstrated that CRP, at concentrations within the reference interval, is a strong predictor of myocardial infarction (1–3), stroke (1, 2, 4), sudden cardiac death (5), and peripheral arterial disease (6) in apparently healthy adults. High-sensitivity methods that are capable of reliably measuring CRP concentrations \( \leq 0.15\) mg/L (approximately the first and second percentiles of CRP distribution in healthy adults) have, therefore, been developed (7, 8). Furthermore, an algorithm for risk assessment of future coronary events that combines both CRP concentration and total cholesterol:HDL-cholesterol ratio has been proposed (9). According to this algorithm, an individual’s risk can be estimated with use of quintiles of CRP and lipids derived from ongoing population-based surveys.

Because high-sensitivity methods have only recently become available, the frequency distributions of CRP in apparently healthy US adults have not been carefully examined. Such information is useful in determining the most appropriate CRP cutpoints for the risk assessment algorithm in men and women. In this report, we describe