Parathyroid Hormone Is More Stable in EDTA Plasma Than in Serum, Paul Glendenning,* Leonie L. A. Laffer, Hayley K. Weber, Alexander A. Musk, and Samuel D. Vasikaran (Department of Core Clinical Pathology and Biochemistry, Royal Perth Hospital, Perth 6000, Western Australia; * author for correspondence: fax 61-8-9224-2491, email Paul.Glendenning@health.wa.gov.au)

Measurements of circulating parathyroid hormone (PTH) are important in the evaluation of individuals with hyper- or hypocalcemia and for the investigation of PTH function in bone and mineral disorders (1, 2). Serum may be a good alternative to EDTA plasma in the assessment of PTH status and is an acceptable alternative to EDTA plasma in the reformulated IMMULITE 2000 intact PTH (iPTH) assay. However, reports on the stability of PTH in serum and EDTA plasma are conflicting (3–5).

Blood samples from 36 patients were collected in parallel into 10-mL tripotassium EDTA tubes, ensuring complete sample filling of the collection vessel (6), and into Becton Dickinson SST II gel separator tubes. PTH was assayed with the reformulated IMMULITE 2000 iPTH assay (beginning with lot L2KPP 106) within 3 h of collection and reassayed on both samples after storage for 3 days at room temperature.

The mean iPTH was 14.8 pmol/L (range, 0.9–27 pmol/L) for EDTA-plasma samples at baseline (target value). Serum iPTH values were 35% lower. After 3 days of storage at room temperature, the serum iPTH values were >60% lower than the baseline EDTA-plasma target values (Fig. 1A), whereas the iPTH in EDTA was marginally higher (by 8%). Statistical analysis (repeated-measures ANOVA and Dunnett’s post hoc test) of all results indicated that serum values both at baseline and after 3 days were statistically lower than the target value (P <0.0001). In contrast, there was no significant difference between EDTA-plasma values at baseline and after 3 days at room temperature. The magnitude of difference between serum and EDTA-plasma iPTH was similar for high iPTH values and iPTH values within the reference interval (Fig. 1B).

The Royal Australasian College of Pathologists Quality Assurance Program determines the allowable limits of performance for iPTH assays by two criteria: (a) <25% difference between the sample and the target value when iPTH is >10 pmol/L; and (b) 2.5 pmol/L absolute difference between the sample and target value when the target value is <10 pmol/L. When the Royal Australasian College of Pathologists Quality Assurance Program criteria were applied, 19 of 36 (52%) serum samples at baseline and 27 of 36 (75%) serum samples stored at room temperature for 3 days failed assurance criteria, in contrast to one EDTA sample stored at room temperature for 3 days.

We defined our reference interval for iPTH (0.8–8.0 pmol/L) according to the values in healthy, vitamin D-sufficient blood donors (7). When we applied this reference interval for iPTH as the diagnostic classification criterion, 6 of 36 (17%) serum samples at baseline and 13 of 26 (50%) serum samples stored at room temperature for 3 days were misclassified, in contrast to two EDTA samples stored at room temperature for 3 days.

Despite attempts to analyze serum samples expeditiously, serum values for iPTH were significantly lower than in EDTA-plasma samples. Thus, the IMMULITE 2000 iPTH assay does not give comparable results for serum and EDTA plasma. The further decline in iPTH values in serum at 3 days is consistent with the susceptibility of PTH to degrade in serum samples. As long as adequate sample volume during collection is ensured, EDTA samples are the most appropriate for iPTH mea-
Denaturing Gradient Gel Electrophoresis for the Molecular Characterization of Six Patients with Guanidinoacetate Methyltransferase Deficiency, Chike B. Item, Carmen Stromberger,1 Adolf Mühl,1 Claudia Edlinger,1 Olaf A. Bodamer,1 Andreas Schulze,2 Robert Surtees,2 Vincenzo Leuzzi,4 Gajia S. Salomons,5 Cornelis Jakobs,5 and Sylvia Stöckler-Ipsiroglu6 (1 Department of Pediatrics, University Hospital and General Hospital (AKH), Währingerstrasse 18-20, A-1090 Vienna, Austria; 2 Department of Pediatrics, University Hospital, D-69120 Heidelberg, Germany; 3 Institute of Child Health, University College, WC1N 2AP London, United Kingdom; 4 Dipartimento di Scienze Neurologiche e Psichiatriche dell’Éta Evolutiva, Università “La Sapienza”, 00185 Rome, Italy; 5VU University Medical Center, De Boelelaan 1117, 1007 Amsterdam, The Netherlands; *author for correspondence: fax 43-1-4063484, e-mail stockler@metabolic-screening.at)

Guanidinoacetate methyltransferase (GAMT); EC 2.1.1.2] deficiency is the first recognized inborn error of creatine biosynthesis, manifesting in infancy with severe neurologic symptoms such as epilepsy, mental retardation, muscular hypotonia, and progressive extrapyramidal movement disorder (1). Patients with GAMT deficiency have shown favorable responses to oral supplementation of creatine-monohydrate, but complete reversal of symptoms has not been observed (2). Biochemical findings include high urinary excretion of guanidinoacetate (the immediate precursor of creatine and substrate of GAMT), low urinary excretion of creatinine [conversion product of intracellular creatine; see Ref. (3)], and depletion of creatine in brain and muscle (4). After assessing two index patients (5, 6), we aimed to establish methods for the noninvasive molecular diagnosis of GAMT deficiency. We recently developed a radiochemical method for the determination of GAMT activity in cultured skin fibroblasts and in virus-transformed lymphoblasts (7). Here we report a denaturing gradient gel electrophoresis (DGGE) technique for the screening of mutations in the GAMT gene in DNA extracted from dried-blood spot filter-paper samples and from fibroblasts and virus-transformed lymphoblasts.

Three index patients with mutations confirmed by techniques other than DGGE [P1, P2, and P6; see Refs. (5, 8)] and three new patients (P3, P4, and P5) with undetectable GAMT activity but unknown GAMT deficiency mutations (9, 10) were investigated. Details of the patient studies are summarized in Table 1, and the locations and frequencies of the mutations identified in these patients are shown in Fig. 1.

We extracted DNA from dried-blood spot, filter-paper samples and from fibroblasts and lymphoblasts using Chelex-100 particles [Promega; see Ref. (11)] and the Nucleospin C+T reagent set (Machery, respectively). Primer pairs for nested PCR are available as a data supplement at Clinical Chemistry Online (Table 2; http://www.clinchem.org/content/vol48/issue5). Seven DNA fragments were amplified, including exon 1/promoter, the 3′-untranslated region (UTR), exons 2–6, and adjacent intron boundaries. The first round of PCR was carried out in a mixture containing 500 ng of DNA extracted from dried-blood spots, 800 nM each outside primer, 10 mM Tris-HCl (pH 8.3), 1.5 mM KCl, 2 mM MgCl2, 200 μM each deoxyribonucleotide, and 2.5 U of Ampli-Taq Gold DNA polymerase in a total reaction volume of 50 μL. Alternatively, 100 ng of DNA extracted from fibroblasts and lymphoblasts and 400 nM each of the outside primers were used. PCR conditions for a Perkin-Elmer-Cetus DNA Thermal Cycler were as follows: denaturation at 95 °C for 5 min followed by 40 cycles at 95 °C for 1 min, 65 °C for 1 min, 72 °C for 1 min, and a final elongation at 72 °C for 10 min. In a second PCR round, thermal cycling was carried out for 35 cycles as above with 0.5 μL of PCR product from the first round of amplification with 400 nM each of the nested primers.

DGGE was carried out essentially as described previously (12) with 20 μL of sample loaded onto a 10% polyacrylamide gel containing a 40–70% denaturing gradient (exon 2b); 8% polyacrylamide gel containing a 25–65% or a 40–70% denaturing gradient (exons 3, 4, 5, and 6); 12% polyacrylamide gradient gel containing a 40–80% denaturing gradient (exon 2a, 3′-UTR). A 100% denaturing gradient gel contains 7 mol/L urea and 400 mL/L (by volume) deionized formamide.

References
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