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Influence of Preanalytical Factors on the Immulite Intact Parathyroid Hormone Assay

To the Editor:

Preanalytical factors that affect parathyroid hormone (PTH) concentrations are not well defined. Measured PTH concentrations in EDTA plasma may (1, 2) or may not (3) differ from those in serum. PTH has been reported variously to be stable for hours to days (1–6). In addition, different PTH assays cross-react to various extents with biologically inactive, N-terminally truncated PTH molecules (7) that may have different stabilities. The specificity of a given immunometric assay depends on the binding sites of the anti-PTH antibodies used. So-called “intact” PTH (iPTH) assays show cross-reactivity with peptides lacking N-terminal amino acids. Assays measuring only PTH with intact NH₂ termini are named CAPTM; whole PTH, 3rd generation; or bioactive PTH assays (7).

In 2001, DPC introduced a reformulated iPTH sandwich assay (starting with lot no. 106) in which the polyclonal tracer antibody had been replaced by a monoclonal antibody raised against an epitope within the first 34 amino acids of PTH. With this new method, reference intervals and, in particular, PTH concentrations measured in serum samples of dialysis patients are lower (data not shown). In March 2003, DPC reported underrecovery of PTH with this method (8), and a new lot of raw materials was introduced, starting with assay lot no. 112. The calibra-

tion for the Immulite analyzer was adjusted at assay lot no. 113 (8). Shortly before this, we had found that PTH concentrations in EDTA plasma measured with lot no. 109 apparently increased with time (data not shown), whereas a previous report (1) indicated that PTH as measured by this method was stable in EDTA plasma (1). The changes in the PTH assay and the conflicting results on PTH stability in EDTA samples led us to investigate with the newer monoclonal/polyclonal iPTH assay both PTH reference intervals and stability as well as agreement between the PTH measurements on the Immulite and Immulite 2000 analyzers.

Reference intervals were estimated by the nonparametric method in Analyze-It for Microsoft Excel, based on results for material from nonpregnant volunteers from the outpatient clinic (age range, 20–65 years). Individuals suffering from renal failure or taking vitamin and/or mineral supplements were not included in the study. All samples were obtained between 0900 and 1300. Almost all volunteers (98%) were Caucasians; 61% were female, and 39% were male. Blood was collected into two dipotassium EDTA VacutainerTM tubes (plastic; BD) and into a serum tube with clot activator and SSTIITM gel. Heparin plasma was not included in the study because measurement of PTH in heparin plasma frequently led to inexplicable outliers (data not shown). After venipuncture, one EDTA tube was immedi-

ately put into melting ice (EDTAice), the other EDTA and the serum tube were kept at room temperature. Serum tubes were allowed to clot before centrifugation. EDTAice plasma was centrifuged at 4 °C. PTH was assayed on an Immulite 2000 (assay lot no. 112) and an Immulite (assay lot no. 113) analyzer within 2.5 h of venipuncture, according to the manufacturer's protocols.

Of the 137 volunteers, 9 had PTH concentrations more than 1.5 interquartile ranges from the first quartile above the median and were investigated as possible outliers. All nine samples had pathologically low calcium concentrations or increased plasma phosphate or creatinine concentrations and thus were excluded from the reference interval study. To assess the stability of PTH, we retested samples that had been stored as serum and plasma at 4 °C on the Immulite 2000 48 h after the initial measurements.

PTH measured in serum was lower than in EDTA or EDTAice plasma ($P < 0.0001$, Wilcoxon signed-ranks test). For comparison Table 1 also contains results from a similar reference interval study we had conducted previously with lot no. 109. The PTH concentrations found with lot no. 112 on the Immulite 2000 were slightly higher than in the reference interval study with lot no. 109. When we used the same samples, the reference intervals determined with the Immulite were higher than those determined with the Immulite 2000.

Table 1. Median PTH concentrations and estimated nonparametric 95% reference intervals obtained on Immulite and Immulite 2000 analyzers.

Analyzer	PTH assay lot no. ^a	No. of samples	Material	Median, pmol/L	Reference interval, pmol/L
Immulite	113	124	Serum	3.5 ^b	1.4–6.3
		124	EDTA	4.6	2.0–8.3
		124	EDTAice	3.9	1.5–7.8
Immulite 2000	112	128	Serum	2.8 ^b	1.2–5.2
		128	EDTA	3.8	1.6–7.0
		128	EDTAice	3.3	1.4–6.1
Immulite 2000	109	134	Serum	2.1 ^b	0.7–4.9
		121	EDTA	2.7	0.9–5.8
		135	EDTAice	2.4	0.6–5.2

^a Lot nos. 112 and 113 are “improved” lots of the monoclonal/polyclonal assay reagent sets, whereas lot no. 109 is from the original monoclonal/polyclonal assay reagent set.

^b $P < 0.0001$ vs corresponding EDTA or EDTAice samples (Wilcoxon signed-ranks test).

Regression analysis incorporating all serum samples measured on both analyzers revealed a proportional difference of 21% for the Immulite analyzer ($n = 135$; $r = 0.97$, Spearman rank correlation). Samples measured in EDTA or cooled EDTA plasma on the Immulite showed differences of 15% and 19%, respectively ($n = 135$; $r = 0.97$ and 0.96 , Spearman rank correlation), compared with the Immulite 2000.

Finally, we tested the *in vitro* stability of PTH by measuring PTH before and after storage of serum and plasma for 48 h at 4 °C. Serum PTH decreased from a median concentration of 2.6 pmol/L to 2.4 pmol/L within 48 h ($P = 0.0003$, Wilcoxon test). PTH in EDTA plasma increased marginally, from 3.8 pmol/L to 4.1 pmol/L ($P = 0.008$, Wilcoxon test), and PTH in ice-cold EDTA plasma remained constant at ~3.2 pmol/L ($P = 0.52$, Wilcoxon test). The quality-control data indicated that there was no obvious drift. Although for serum and EDTA plasma the changes in PTH concentrations with time are statistically significant, the clinical relevance of the changes is questionable.

The results presented here confirm previous reports describing higher apparent PTH concentrations in EDTA plasma than in serum (1,2) and changes in PTH concentrations with time (1–6). In agreement with what has been published for the DPC PTH assay (1), we found a slight increase in PTH concentration in EDTA plasma with time. The decrease in serum PTH concentrations was much less profound after 2 days (this study) than after 3 days (1) after venipuncture. This may be attributable to the fact that in the study of Glendenning et al. (1) the samples were stored at room temperature, whereas we stored them, similar to patient material, at 4 °C. The observation that PTH does not decrease in EDTA plasma at room temperature may be attributable to chelation by EDTA of cations essential to (metallo)protease activity.

In conclusion, reference intervals for PTH are dependent on sample type, and reports on the stability of

PTH in serum and plasma are inconsistent. Therefore, within one laboratory, PTH should be determined in one type of sample only and as soon as possible after venipuncture. Excess EDTA in an underfilled tube can inhibit signal production in methods that use alkaline phosphatase; for such methods, serum should be the material of choice. Laboratories measuring PTH on both the Immulite and Immulite 2000 analyzers should make sure that the analyzers give similar results for patient material.

DPC Nederland (Breda, The Netherlands) kindly provided the PTH reagents used in this study.

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Editor's Note: A representative of the manufacturer declined to send a reply for publication, but indicated in comments to us that the letter is accurate and that the problem has been rectified.

Proteinase K Added to the Extraction Procedure Markedly Increases RNA Yield from Primary Breast Tumors for Use in Microarray Studies

To the Editor:

Gene expression profiling by microarray technology has demonstrated the possibilities of biological separation, prognostication, and prediction (1,2). In this study we demonstrate that the addition of proteinase K to the extraction procedure improves the yield of RNA from primary breast tumors, making the majority of samples eligible for array analysis by Qiagen RNeasy Minikit. In addition, we investigated whether the addition of proteinase K has any influence on the gene expression profile.

The Qiagen RNeasy Mini protocol was used as described by the manufacturer (3,4) for extraction of total RNA from 209 frozen primary breast tumors from women who had undergone surgery for breast cancer at Karolinska Hospital. The study was approved by an ethics committee at the hospital. Biopsies from the same tumors were extracted either with or without proteinase K treatment as follows. Each sample (maximum of 40 mg) was cut into smaller pieces and homogenized for 30–40 s in 400 μ L of RLT lysis buffer containing mercaptoethanol with use of a polytron PT1200 homogenizer (Kematic AG). For the proteinase K-sample, one volume (400 μ L) of 700 g/L ethanol was added to the homogenate, with mixing during the addition, after which the sample was applied to a RNeasy mini spin column. For the proteinase K+ sample, 785 μ L of doubly distilled water plus 13.5 μ L of proteinase K (20 g/L; Qiagen GmbH) was added to the homogenate, which was then mixed and incubated for 10 min at 55 °C.