Influence of preanalytical factors on the immulite intact parathyroid hormone assay

Citation for published version (APA):
https://doi.org/10.1373/clinchem.2003.027912

DOI:
10.1373/clinchem.2003.027912

Document status and date:
Published: 01/01/2004

Document Version:
Publisher’s PDF, also known as Version of Record (includes final page, issue and volume numbers)

Please check the document version of this publication:
• A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
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Download date: 26. Oct. 2023
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 iPPTH assays show cross-reactivity with peptides lacking N-terminal amino acids. Assays measuring only PTH with intact NH2 termini are named CAP™; whole PTH, 3rd generation; or bioactive PTH assays (7).

In 2001, DPC introduced a reformulated iPPTH sandwich assay (starting with lot no. 106) in which the polyclonal tracer antibody had been replaced by a monoclonal antibody raised against an epitope within the first 34 amino acids of PTH.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-

<table>
<thead>
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<th>Analyzer</th>
<th>PTH assay lot no.</th>
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<th>Material</th>
<th>Median, pmol/L</th>
<th>Reference interval, pmol/L</th>
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<td>128</td>
<td>Serum</td>
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<td>0.6–5.2</td>
</tr>
</tbody>
</table>

* 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.

References

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DOI: 10.1373/clinchem.2003.027912

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 μL of RLT lysis buffer containing mercaptoethanol with use of a polytron PT1200 homogenizer (Kinematica AG). For the proteinase K–sample, one volume (400 μL) 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 μL of doubly distilled water plus 13.5 μL 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.