Influence of preanalytical factors on the immulite intact parathyroid hormone assay

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providing details and we will investigate your claim.
In March 2003, DPC re-assayed patients are lower (data not shown). After 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 re-tested 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 EDTA ice (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>
<thead>
<tr>
<th>Analyzer</th>
<th>PTH assay lot no.*</th>
<th>No. of samples</th>
<th>Material</th>
<th>Median, pmol/L</th>
<th>Reference interval, pmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immulite</td>
<td>113</td>
<td>124</td>
<td>Serum</td>
<td>3.5±</td>
<td>1.4–6.3</td>
</tr>
<tr>
<td></td>
<td>113</td>
<td>124</td>
<td>EDTA</td>
<td>4.6</td>
<td>2.0–8.3</td>
</tr>
<tr>
<td></td>
<td>113</td>
<td>124</td>
<td>EDTAice</td>
<td>3.9</td>
<td>1.5–7.8</td>
</tr>
<tr>
<td>Immulite 2000</td>
<td>112</td>
<td>128</td>
<td>Serum</td>
<td>2.8±</td>
<td>1.2–5.2</td>
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<tr>
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<td>112</td>
<td>128</td>
<td>EDTA</td>
<td>3.8</td>
<td>1.6–7.0</td>
</tr>
<tr>
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<td>112</td>
<td>128</td>
<td>EDTAice</td>
<td>3.3</td>
<td>1.4–6.1</td>
</tr>
<tr>
<td>Immulite 2000</td>
<td>109</td>
<td>134</td>
<td>Serum</td>
<td>2.1±</td>
<td>0.7–4.9</td>
</tr>
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<td>109</td>
<td>134</td>
<td>EDTA</td>
<td>2.7</td>
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<td></td>
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<td>EDTAice</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 (metal-)

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

References

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 Qiaqen RNasey Miniikit. In addition, we investigated whether the addition of proteinase K has any influence on the gene expression profile.

The Qiagen RNasey 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 (Kine
matica 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 RNasey 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.

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

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