We compared different DNA amounts and several DNA sources. Variant DNA amounts ranging from 9 to 640 ng of DNA template from either blood or serum gave reproducible genotyping results. The latter indicates the high flexibility of the method and allows for the omission of DNA quantification before genotyping.

We conclude that this assay is a valuable method that allows simple and rapid high-throughput genotyping in the clinical laboratory.

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References

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Biological Variation in Serum Type I Collagen Carboxy-Terminal Telopeptide Concentrations

To the Editor:
Roche Diagnostics recently automated (1) (on the Elecsys® analyzer) the β-CrossLaps™ assay (2) for measurement of bone-derived degradation products of type I collagen carboxy-telopeptide in serum (s-βCTX). However, information on the biological variation of s-βCTX is lacking, a fact that is limiting because the clinical utility of bone markers can be significantly affected by their physiological variations (3).

To investigate the biological variation of s-βCTX, we took four blood specimens from each of 10 healthy volunteers (5 men and 5 premenopausal women; ages 25–50 years) on the same day once a week for 4 weeks. “Healthy” subjects were studied to ensure that any fluctuation in s-βCTX concentrations could truly be attributed to biological variation and were not caused by pathological variations in bone homeostasis. In accordance with the Helsinki II Declaration, the design and execu-
tion of the experiment were explained thoroughly to the subjects, and informed consent was obtained. The inclusion criteria were that the subjects be within 20% of ideal body weight, have no bone or connective tissue disorder, and for women, have regular menstrual cycles and no use of hormonal contraceptives. In addition, none of the subjects took any medication or consumed substantial quantities of alcohol.

Venous blood was obtained between 0800 and 0900 from subjects who had fasted for 12 h and had not smoked or exercised that morning. Samples were collected by the same phlebotomist using vacuum collection tubes with minimal stasis while subjects were in the sitting position. Serum specimens, separated by centrifugation, were stored at -25°C until analysis. When all of the specimens were available, they were thawed, mixed, centrifuged, and analyzed in a single run in duplicate in random order. s-CTX was measured using the β-CrossLaps/Serum assay on the Elecsys 2010 analyzer.

The analytical (CV_A), within-subject (CV_I), and between-subject (CV_G) components of variation were calculated by nested ANOVA from replicate analyses (4). In particular, CV_A was estimated from the duplicate results for each specimen, CV_I from the serial results for each subject, and CV_G from the total variance of the data minus the analytical and intraindividual components. The desired analytical imprecision; the index of individuality (CV_I/CV_G), which yields information about the utility of conventional population-based reference intervals; the critical difference (2.77 CV_A^2 + CV_G^2)^1/2, i.e., the minimal significant difference (P < 0.05) between two consecutive measurements of the marker in the same patient; and the number of specimens that should be collected to estimate (P < 0.05) the homeostatic set point of an individual within ± 10% [1.96^2(CV_A^2 + CV_G^2)/100] were also estimated (Table 1) (4).

Although the mean s-CTX values showed no sex-related difference, women had a lower within-subject variability and a higher intraindividual fluctuation. This marked individuality of s-CTX in women, reflected by the low index of individuality, suggests that individuals may have values that are highly unusual for them but still within a population-derived reference interval, making the use of conventionally calculated reference limits inadequate for interpretation of s-CTX values in females. The results of recent analyses for an individual may best be used as a guide to possible pathology in that individual. Conversely, given the lower CV_G and the high index of individuality in men, the use of reference limits may be of value in assessing unusual s-CTX results in male patients.

The desirable analytical imprecision for s-CTX, taken to be ≤0.5 CV_I was ≤8.5%. Thus, in this limited assessment, the intrabatch imprecision (CV_A) of the Elecsys CrossLaps assay met this goal. The critical difference was 51% for all subjects and 40% for women. Because preliminary reports have shown mean changes of 60% or more in s-CTX concentrations in females post treatment (2), our results suggest that s-CTX may be of use in monitoring response to therapy in individual patients. Thirteen samples are required to estimate an individual’s s-CTX value to within 10% of the true mean value. Clearly, it is impossible to obtain in clinical practice enough samples from a patient to estimate that individual’s true s-CTX value, but at least two samples should be obtained in the baseline evaluation of a subject to significantly reduce the effect of biological variability on the estimation of s-CTX concentrations.

We thank Francesca Stefini for skillful technical assistance and Roche Diagnostics for the gift of reagents to carry out the study.

References

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Evaluation of the AxSYM Homocysteine Assay and Comparison with the IMx Homocysteine Assay

To the Editor:

Increased plasma total homocysteine (tHcy), a sulfur amino acid, is an important risk factor for vascular disease (1) and for arterial and venous thromboembolism (2). Several techniques have been developed to measure plasma or serum tHcy concentrations (3). Given the extension of this determination to routine clinical chemistry laboratories, an immunoassay has been developed for use with the AxSYM analyzer (4). The AxSYM is a completely automated

Table 1. Mean values, estimated average analytical (CV_A), intraindividual (CV_I), and interindividual (CV_G) variations, and derived indices for s-CTX.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean, µg/L</th>
<th>CV_A, %</th>
<th>CV_I, %</th>
<th>CV_G, %</th>
<th>II*, %</th>
<th>CD, %</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>0.34</td>
<td>7.8</td>
<td>17</td>
<td>40</td>
<td>0.41</td>
<td>51</td>
<td>13</td>
</tr>
<tr>
<td>Men</td>
<td>0.32</td>
<td>21</td>
<td>10</td>
<td>2.10</td>
<td>0.21</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>Women</td>
<td>0.36</td>
<td>12</td>
<td>56</td>
<td>0.21</td>
<td>40</td>
<td>13</td>
<td>8</td>
</tr>
</tbody>
</table>

*a, index of individuality; CD, critical difference; n, number of specimens that should be collected to estimate the homeostatic set point of an individual.