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 interindivi-

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>63</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>0.36</td>
<td>12</td>
<td>56</td>
<td>0.21</td>
<td>40</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

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

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.

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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
system, which allows immediate and continuous access. We evaluated the analytical performance of the automated AxSYM assay and compared it with the IMx Hcy assay (5), a method widely used for tHcy determination in laboratories. Both methods use reduction of protein-bound Hcy by dithiothreitol, conversion of Hcy to S-adenosylhomocysteine in the presence of adenosine catalyzed by hydrolase enzyme and detection of S-adenosylhomocysteine by fluorescence polarization immunoassay.

The AxSYM assay, however, gives the first result within 35 min (rather than 60 min) and allows random access. The AxSYM assay does not require sample pretreatment, and serum or plasma may be used. The assay requires 108 µL of sample, and up to 60 samples can be processed in 1 h (20 for the IMx).

The linearity of the method was confirmed up to 50 µmol/L (i.e., the highest calibrator concentration). Linearity was assessed by serially diluting (1:2, 1:4, 1:8, 1:16, and 1:32) three high-tHcy concentration plasmas (>40 µmol/L) with AxSYM buffer. Sample dilutions were processed in duplicate. The results (in percentage of theoretical concentrations) showed values between 96.8% and 103.2%. Linearity was not tested above 50 µmol/L, which is the upper limit allowed by the software based on the calibrator values. To determine the limit of detection, we analyzed 10 replicates of the A calibrator (0 µg/L) and 2 replicates of the B calibrator (2.5 µmol/L) in four runs (6). The average limit of detection was 0.3 µmol/L.

The three controls [low (7 µmol/L), medium (12.5 µmol/L), and high (25 µmol/L)] for the Abbott IMx system were analyzed in replicates of 20 over 3 consecutive days. Within-run CVs [3.5% (low), 2.7% (medium), and 2.3% (high)] were satisfactory and similar to those reported for the IMx assay (7). To determine between-day imprecision, each of these three controls was analyzed over 20 different consecutive days: CVs were 3.8% (low), 2.9% (medium), and 2.3% (high), indicating a good precision for the method. The addition to two normal plasmas of physiological and 10-fold physiological concentrations of methionine (30 and 300 µmol/L), cysteine (50 and 500 µmol/L), or cystine (70 and 700 µmol/L) showed tHcy results 99.4% of baseline after methionine addition, 96.2% after cysteine addition, and 102.4% after cystine addition.

Plasma samples from 100 patients were analyzed by both the AxSYM and IMx tHcy assays during a 10-week period. All of these patients were scheduled for routine tHcy determination, and clinical practice complied with the ethics rules of our institution. Plasma samples were stored at −20 °C before assay and were analyzed simultaneously on both analyzers. Deming regression (see Fig. 1) showed an excellent agreement between the two methods (y = 1.04x − 0.05; r = 0.99; P < 0.001; 

\[ y = 0.8964; 95\% \text{ confidence interval for the intercept, } 0.85; 5 \]

\[ y = 1.04x - 0.05; r = 0.99; P < 0.001; \]

In conclusion, the tHcy AxSYM method shows good accuracy and precision and correlates highly with the IMx assay. Furthermore, the AxSYM method is less time-consuming and benefits from the analyzer’s random/continuous mode. The tHcy AxSYM assay is therefore suitable for routine use in the clinical laboratory.

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References

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