benzodiazepine confirmation tests compared to approximately 20 tests during a similar 3-month period in 2006. Because of the unacceptably high false-positive rates for benzodiazepine screening using the Multigent reagents and the significant increase in toxicology workload and costs, the VA Boston Healthcare System elected to switch to the Abbott/SYVA reagents for benzodiazepine screening in August 2007. It is our understanding that Abbott is aware of the problem we have described and is in the process of reformulating its Multigent benzodiazepine reagent to increase its specificity.

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Reference


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Table 1. Summary of historical and recent experience for screening and confirmation testing for benzodiazepines in urine.

<table>
<thead>
<tr>
<th></th>
<th>Total screened</th>
<th>Positive, n</th>
<th>Positive, %</th>
<th>Confirmed positive, n</th>
<th>Confirmed positive, %</th>
<th>False-positive rate, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000 (6 months)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzodiazepine screens&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11 115</td>
<td>1451</td>
<td>13.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzodiazepine confirmations&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1451</td>
<td>1372</td>
<td>94.6</td>
<td>5.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2007, June and July</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzodiazepine screens&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2447</td>
<td>615</td>
<td>25.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzodiazepine confirmations&lt;sup&gt;d&lt;/sup&gt;</td>
<td>615</td>
<td>457</td>
<td>74.3</td>
<td>25.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> SYVA™ EMIT™ reagents on Olympus AU640™ instrumentation.
<sup>b</sup> HPLC confirmation on Biorad REMEDI™.
<sup>c</sup> Abbott Multigent™ reagents on Abbott Architect™ instrumentation.

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Are Clinical Laboratories Prepared for Accurate Testing of 25-Hydroxy Vitamin D?

To the Editor:

Vitamin D deficiency is an important concern, but assays for serum 25-hydroxy vitamin D (25-OH-D),<sup>1</sup> the accepted marker for vitamin D nutritional status, are not standardized. A quick search of Medline yielded 6014 citations on vitamin D deficiency, 51 of which were published in the first 6 mo of 2007 (1). Recognition that vitamin D deficiency may be more prevalent in most patient populations than earlier assumed has resulted in an unexpected and marked increase in the volume of testing for 25-OH-D in clinical laboratories (1). Historically, not many tests in the clinical laboratories have increased at the rate of 80% to 90% per year, as is the case for 25-OH-D in our reference laboratory. Several million 25-OH-D tests are likely to be performed this year in the US by various reference and local hospital laboratories, raising questions about the adequacy of the methods used to make these measurements.

Various methods are available for measuring circulating concentrations of 25-OH-D (until recently, only RIA was available). Current methods include HPLC, RIA with low throughput to high throughput, automated chemiluminescence immunoassays, and liquid chromatography-tandem mass spectrometry (LC-MS/MS). These new methods have already aroused controversy (2–4). Correlation and agreement studies between immunoassays and LC-MS/MS methods for 25-OH-D have been reported by several investigators (2–4). These studies report reasonable correlations but with significant differences, the
reasons for which are not transparent or well understood. Automated or manual competitive immunoassays are known to have less specificity for low-molecular-weight compounds, and immunoassays for 25-OH-D are no exception.

The College of American Pathologists (CAP) and the UK-based DEQAS (Vitamin D external quality assessment scheme) surveys provide independent approaches to monitor the performance of laboratories that use various methods for testing of 25-OH-D. The survey feedback does not assess the accuracy of 25-OH-D measurements by laboratories, but scores laboratories for agreement within the group using a particular method. Recent CAP data (CAP survey, 2006 Ligands Special) indicate that clinical laboratories using chemiluminescence immunoassays can report a result ranging from 41 to 96 μg/L for a survey sample with a value of 31 μg/L determined by LC-MS/MS (BGS-04 in Fig. 1). There could be many reasons for these variations, including drifts in the reagents being manufactured, but there is a clear and urgent need for harmonization and standardization.

NIST is developing additional quality control materials (human serum, SRM 972) that will contain 25-OH-D2, 25-OH-D3, and the metabolite 3-epi-25-OH-D at 4 different concentrations as characterized by LC-MS/MS. Although the biological significance of the metabolites remains to be elucidated, the preparation of this SRM is especially important for assays for which the cross-reactivity with these metabolites is not well defined (5).

LC-MS/MS is becoming the technique of choice for various reference laboratories. Laboratories that use in-house LC-MS/MS have responsibility for many steps of the assay. The LC-MS/MS technology for testing of human samples is not approved by the FDA, and manufacturers of LC-MS/MS instrumentation are not responsible for troubleshooting the assays. Laboratories performing 25-OH-D testing by LC-MS/MS technology have differences in their standard operating procedures, and thus interlaboratory CVs are in the range of 20%. The preparation of the reagents required for in-house LC-MS/MS assays is conducted by individual laboratories under their institutionally regulated standard procedures. The complexity of the LC-MS/MS technology in its present form demands a robust, fully automated platform that can meet the need for throughput, precision, and accurate testing of vitamin D and metabolites. Multiplexed immunoassays may have the potential of achieving accuracy and precision for multiple vitamin D metabolites. For better patient care, the goal should be not only to have an accurate 25-OH-D value but also precision for 25-OH-D testing, with a CV <1%.

Currently there are no guidelines or agreement among clinical laboratories on the optimum reference intervals for 25-OH-D to classify patients with moderate to severe vitamin D deficiency. Before it is too late, it is in the interest of clinical laboratories and diagnostic companies to work with each other to standardize the reagents and reference intervals to achieve the quality for 25-OH-D tests that our patients deserve.

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References
1 Nonstandard abbreviations: MPO, myeloperoxidase; CRP, C-reactive protein.


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Biological Variation of Myeloperoxidase

To the Editor:

Myeloperoxidase (MPO)

Myeloperoxidase (MPO)

To the Editor: Myeloperoxidase (MPO) has been shown to play a role in the pathogenesis of coronary artery disease. It is released from activated neutrophils at sites of vascular damage and has been shown to increase in atherosclerotic plaques before the onset of myocardial injury (1). Elevations in MPO occur independently of C-reactive protein (CRP) and other markers of inflammation, and it has been suggested as a marker of risk in patients who have high as well as low cardiac troponin T concentrations and who present with acute coronary syndrome (2).

We recruited 12 apparently healthy individuals, 10 women and 2 men with a mean age of 33 years (range 27–43) for estimation of the biological variation of MPO. None had a history of heart disease or any clinically apparent inflammatory processes. Serum samples were collected between 0800 and 1000 on the same day of the week, weekly for 5 weeks as described by Fraser and Harris (3). Venous blood was collected in plastic serum separator tubes. All samples were centrifuged after clotting and were stored at −70 °C before analysis. All samples were analyzed with the MPO ELISA kit from Immundagnostik and the model 3550 microplate reader from Bio-Rad.

The statistical methodology of Fraser and Harris was used unless otherwise stated (3). Except for the analytical assessment, all assessments were done on both untransformed and logarithmically transformed (natural logarithm) data. The data, which are presented in Fig. 1, provided 60 estimates of analytical variance and 12 estimates of within-subject variance. The analytical variance plotted against the cumulative percentile ranking followed the expected χ² distribution within good proximity. Cochran’s test of maximum variance identified 1 analytical outlier. The residuals of a 1-way ANOVA of the means of duplicates were used for assessment of within-subject distribution (a deviation from the methodology of Fraser and Harris). Cochran’s test detected 1 within-subject outlier that was not present after log-transformation. The outlier was in a sample from subject 1, from whom the analytical outlier was also obtained. In order not to be biased toward 1 distribution, all values of subject 1 were excluded from further analysis. No other outliers were detected after this exclusion. Nonnormality of the untransformed and log-transformed data (after exclusion of subject 1) was not apparent by Shapiro-Wilk tests (P = 0.58 and P = 0.26, respectively). The within-subject index of heterogeneity was calculated as the ratio of the CV of the individual variances to the theoretical CV calculated as [2/(n − 1)]1/2, where n is the average number of specimens from each subject (3). The absolute difference of this ratio from unity was less than the theoretical SD calculated as 1/(2√n), suggesting that the within-subject variances were homogenous. Nonnormality of the between-subject distribution as assessed using subject averages and the Shapiro-Wilk test could not be shown for either the untransformed or log-transformed data (P = 0.10 and P = 0.09, respectively). No outliers were found in either of the 2 distributions using a Reed-Dixon ratio criterion of 1/3. Given these statistics, the analytical (CVₐ), within-subject (CVₗ), and between-subject (CV₅) CVs were estimated with nested ANOVA of the untransformed data only.

The CVₐ, CVₗ, and CV₅ were 4%, 36%, and 30%, respectively. The design of the biological variation study led to an unrealistically low CVₐ, which is desirable for accurate estimates of the other variance components (3). A more realistic CVₐ of 8.4%, obtained from the literature, was used in further calculations (2). The index of individuality (Iᵢ), calculated as (CVₐ + CVₗ)/CV₅, was 1.5, which is satisfactory for comparison of an observed value to a reference value (3). Contrary to CRP, which has a low Iᵢ, the high Iᵢ of MPO supports the use of a population-based cutoff for risk stratification (4). A cutoff of 350 μL/L has been proposed for risk stratification and is used here only as an example because the assay has not been stan-