We wish to report a serious problem in the ADVIA:Centaur® chemiluminescence assay for cobalamin (Bayer Diagnostics; formerly the ACS:Centaur® assay, Chiron Diagnostics). The problem is urgent for two reasons: (a) our findings suggest that many cobalamin-deficient patients are being missed; and (b) the assay is used by increasing numbers of laboratories, which some time ago abandoned radioisotopic methods and were recently faced with the withdrawal of widely used assays by Abbott Laboratories.

Several months after our hospital’s clinical laboratory introduced the Centaur assay, we noted that the number of subnormal results being reported had declined from ~15–20 to 4–5 per month. When we reasayed two serum specimens that had given subnormal cobalamin results with the Abbott IMX® method earlier and had been stored at ~20 °C, the results obtained with the Centaur method were within reference values.

As a result of these observations, we selected 33 sera from our research collection of specimens that had been assayed with our radioisotope dilution assay (RIDA) and stored at ~20 °C. The methodologic details of the RIDA, which uses pure intrinsic factor as the binding protein, have been published (1), with a reference interval of 140–750 pmol/L established in 332 subjects (2).

Table 1. Cardiac marker concentrations.

<table>
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<tr>
<th>Day</th>
<th>CK, µkat/L</th>
<th>CK-MB, µg/L</th>
<th>TnT, µg/L</th>
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<tbody>
<tr>
<td>Day 0</td>
<td>1000a</td>
<td>2.1</td>
<td>8</td>
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<tr>
<td>Day 0</td>
<td>1600</td>
<td>1.8</td>
<td>9.4</td>
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<tr>
<td>Day 0</td>
<td>1800</td>
<td>2.1</td>
<td>11</td>
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<td>Day 13</td>
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<td>0.08</td>
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<td>Day 18</td>
<td>1330</td>
<td>3.2</td>
<td>14</td>
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<tr>
<td>Day 18</td>
<td>1800</td>
<td>15</td>
<td>0.14</td>
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<tr>
<td>Day 19, before hysterectomy</td>
<td>2.2</td>
<td>14</td>
<td>0.12</td>
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<td>Day 20, 1 day after hysterectomy</td>
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<td>4.9</td>
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<td>Day 22</td>
<td>4.0</td>
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<td>&lt;0.04</td>
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a Time sample was collected.

Table 1. Comparison of cobalamin results obtained with three assays.

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<tr>
<th>No.</th>
<th>RIDA b</th>
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<th>Bio-Rad c</th>
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a Results below the reference interval are shown in bold.
b Reference range, 140–750 pmol/L.
c Manufacturer’s reference range, 156–672 pmol/L.
tests of cobalamin status, such as the deoxuridine suppression test and methylmalonic acid and homocysteine assays. The remaining 11 of the 33 sera had cobalamin concentrations within the reference interval by RIDA and came from patients with normal metabolic test results.

All 33 specimens were assayed in a blinded fashion with the Centaur method in the clinical laboratory. Table 1 shows that the results were, in all but two cases, higher than the RIDA results; the median Centaur-to-RIDA ratio was 1.77 (range, 0.66–94.0). Much more importantly, the Centaur method failed to give low results in 16 of the 22 cobalamin-deficient sera (73% failure rate).

We also showed that the poor performance of the Centaur method was not limited to our clinical laboratory. We gave 12 serum aliquots (8 from cobalamin-deficient patients) to a representative of Bayer Diagnostics for assay in a laboratory of his choosing. The results were very similar, and perhaps worse. Falsely normal values were obtained with the Centaur method in seven of the eight deficient sera (169–292 pmol/L, with one outlying value of >1500, vs the 68–124 pmol/L we had obtained by RIDA).

Finally, we largely confirmed the previous RIDA results with another method and demonstrated that the poor performance of the Centaur method was not attributable to storage effects on the sera. We reassayed in a blinded fashion the 21 available samples (16 of which were from cobalamin-deficient patients) with the Quantaphase II® radioassay (Bio-Rad). Table 1 shows that the Bio-Rad assay failed to identify deficiency in only 2 of the 16 cobalamin-deficient sera (13%) compared with the 73% failure rate with the Centaur assay.

For several reasons, the poor performance of the Centaur assay does not appear to be soluble simply by shifting the reference range. The degree of error varied greatly. Some discrepancies were very large, whereas others were relatively small. In addition, perusal of the College of American Pathologists ligand survey results suggests that the Centaur assay performs adequately in the normal reference range but consistently overestimates concentrations in the low range. For example, one survey from 1999 showed that the mean (± SD) result from responding laboratories for the low-cobalamin specimen (K-11) was 130.7 ± 17.2 ng/L with the Centaur method compared with 71.1 ± 7.9 ng/L for the Bio-Rad Quantaphase II method and 83.7 ± 15.1 ng/L with the IMx method. In contrast, the three methods agreed very closely in their results for two ligand specimens with cobalamin concentrations within the reference range (K-12 and K-13) in the same survey. This suggests that the inaccuracy of the Centaur assay may be confined to the low end of cobalamin values, which is exactly the critical area for clinicians.

The misidentification of 73% of cobalamin-deficient sera as normal by the Centaur method is alarming, given the serious clinical consequences that can arise from failure to identify and treat cobalamin deficiency promptly. We do not yet know the reason for the Centaur assay malfunction. However, it is worth noting in general that published documentation and analysis of the new cobalamin assays, especially since the introduction of chemiluminescence assays, has been meager.

References

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Representatives of Bayer Diagnostics respond:

To the Editor:

The data provided by Carmel et al. (their Table 1) suggest that they might be missing cobalamin-deficient patients because they failed to choose an appropriate medical decision point for their patient population with the Bayer ADVIA Centaur VB12 assay. Although the data suggest a “systematic” bias between the RIDA and the ADVIA Centaur, the dispersion (the standard deviation of the difference between ADVIA Centaur and RIDA results) is equivalent to that for Quantaphase vs RIDA. The ADVIA Centaur-vs-RIDA dispersion is 77 pmol/L, and the Quantaphase-vs-RIDA dispersion is 75 pmol/L; these are essentially the same (P = 0.926 by the F-test). In fact, after correcting for systematic bias, ADVIA Centaur and Quantaphase agree better with each other (dispersion of 65 pmol/L) than does either with RIDA. (We assume that Carmel et al. believe that the Quantaphase performs acceptably because they chose it as the referee assay.) Contrary to the assertion of Carmel et al., their data suggest that they may improve detection of cobalamin deficiency in their laboratory by adjusting their medical decision point (“shifting the reference range”, as they put it).

Two authoritative sources state that the delineation between normal and cobalamin-deficient serum concentrations is poorly defined (1, 2). The ADVIA Centaur VB12 Assay Method Manual corroborates this by providing ranges for both normal and cobalamin-deficient subjects, and these ranges overlap. Choosing the optimal medical decision point is a substantial endeavor. The predictive value of a test depends on disease prevalence in the population being screened—which may vary considerably among laboratories—as well as on the inherent clinical sensitivity and specificity of the test (3). For these reasons, it is impossible for a manufacturer to recommend a medical decision point suitable for all laboratories.