Precision of Assays for Sex-Hormone-Binding Globulin: an I-IRMA Kit and Two Eu-IFMA Kits Compared, C. M. G. Thomas, R. J. van den Berg, and M. F. G. Segers (Dept. of Obstet. & Gynecol., Sint Radboud Hosp., Catholic Univ., NL 6525 GA Nijmegen, The Netherlands)

We compared three "sandwich"-type sex-hormone-binding globulin (SHBG) assays, involving either I or Eu as a label (SHBG[125I]-IRMA and SHBG[Eu3+] IFMA kits, Farmos Diagnostica, Turku, Finland; and the Delfia SHBG kit, LKB Wallac, Turku, Finland), based on assay results for 77 serum samples. The linear-regression lines obtained in all cases possible were not significantly different: IFMA(Farmos) = 0.93 IRMA + 2.5, standard error of estimate (Sxy) = 4.1, r = 0.98; IFMA(LKB) = 0.87 IRMA + 13, Sxy = 6.3, r = 0.96; and IFMA(Farmos) = 0.95 IFMA(LKB) - 4.2, Sxy = 4.4, r = 0.97. Thus no systematic differences in results with the three techniques could be attributed to the different labels used.

To study assay precision, we used frequency distributions, determined from the CVs for duplicate measurements of 84 samples of unknown SHBG content. These data, tabulated below, revealed somewhat better assay precision for the SHBG[125I]-IRMA (for 64% of the duplicate unknowns, CVs were <2%, and <4% for 91%) than for the SHBG[Eu3+]-IFMA (67%–73% of unknowns had CVs ≤6%, and 82%–88% ≤10%).

<table>
<thead>
<tr>
<th>Assay</th>
<th>Kit</th>
<th>n</th>
<th>≤2%</th>
<th>≤4%</th>
<th>≤6%</th>
<th>≤8%</th>
<th>≤10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHBG</td>
<td>IRMA (Farmos)</td>
<td>84</td>
<td>64</td>
<td>91</td>
<td>98</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>SHBG</td>
<td>IFMA (Farmos)</td>
<td>84</td>
<td>30</td>
<td>48</td>
<td>67</td>
<td>77</td>
<td>82</td>
</tr>
<tr>
<td>SHBG</td>
<td>IFMA (LKB)</td>
<td>80</td>
<td>20</td>
<td>54</td>
<td>73</td>
<td>82</td>
<td>88</td>
</tr>
<tr>
<td>FSH</td>
<td>IFMA (LKB)</td>
<td>35</td>
<td>43</td>
<td>74</td>
<td>85</td>
<td>88</td>
<td>91</td>
</tr>
</tbody>
</table>

The conclusion that the less-precise results of the SHBG[Eu3+] IFMA are apparently due to use of the time-resolved fluorescence label is countered by our observations involving the Delfia FSH kit (LKB Wallac): for 74% of the duplicate unknowns, CVs were ≤4%, and ≤6% for 85%, findings in accord with the manufacturer’s precision profile. Thus we conclude that the SHBG[Eu3+] IFMA kits should be better optimized if they are to compete well with the available SHBG[125I]-IRMA kit.

We thank the companies and their representatives for the donation of the test kits.

Automated Method for Phosphohexose Isomerase with a Centrifugal Analyzer, C. M. Huang (Clin. Pathol. Dept., Clin. Center, NIH, Bethesda, MD 20892)

The clinical significance of phosphohexose isomerase (PHI; EC 5.3.1.9) is that it is a valuable biochemical marker in monitoring tumor mass (1) as well as in predicting early response and relapse in various malignancies (2, 3). Manual assay, either colorimetric or spectrophotometric, is time-consuming.

We have automated the procedure for determination of serum PHI activity by using a Cobas-Bio centrifugal analyzer and a kit (355-UV) from Sigma Diagnostics, St. Louis, MO. Instrument settings are as follows:

1. Units: U/L
2. Calculation factor: 3215
3. Standard 1 concn: 0
4. Standard 2 concn: 0
5. Standard 3 concn: 0
6. Limit: 6
7. Temp, °C: 30
8. Type of analysis: 2
9. Wavelength, nm: 340
10. Sample vol, µL: 10
11. Diluent vol, µL: 50
12. Reagent vol, µL: 250
13. Incubation time, s: 300
14. Start reagent vol, µL: 0
15. Time of first reading, s: 300
16. Time interval, s: 20
17. No. of readings: 10
18. Blanking mode: 1
19. Printout mode: 1

The standard curve for the automatic enzymatic assay was linear up to 250 U/L. Results by this method (γ) and those by a manual procedure (x) agreed well: the Deming and regression between the two methods was γ = 0.99x + 1.44 U/L, Sxy = 2.2 U/L, r = 0.99, and n = 31. The within-run precision study with phosphohexose isomerase activities of 46.8 and 135.6 U/L gave CVs of 1.3% and 0.7%, respectively. The between-run precision (at 48.0 and 149.7 U/L) was 2.8% and 2.9%, respectively. There was no significant interference from triglyceride concentrations as great as 10.0 g/L. Total bilirubin showed an interference of 0.2 U/L per milligram of bilirubin in the range of 0–96 mg of bilirubin per liter. Erythrocytes caused an analyte-dependent and an analyte-independent interference. The positive bias by erythrocytes was calculated to be 5.05 × 0.001 × no. of erythrocytes for the analyte-independent interference. The analyte-dependent increment by erythrocytes was 0.013 × 0.001 × no. of erythrocytes × PHI activity (U/L). Therefore, hemolyzed sera should not be used for measuring the activity of this enzyme.

This assay procedure is inexpensive; currently, its estimated cost is $0.30 per test. It takes 20 min to assay 25 specimens in a single run, compared with the 200 min required by the manual procedure. This method makes the