from 25 to 300 μmol/L yielded the equation: \( y = 0.96x - 1.37, r = 0.984, \text{SEE} = 9.16 \).

Reference interval. We used the plasma ammonia results obtained from volunteer hospital personnel to establish the reference values for this group. By the nonparametric percentile technique described by Herrera (11) and Reed et al. (11), the central 95 percentile interval is 16–35 μmol/L. This agrees reasonably well with ranges reported for the manual technique (5–69 μmol/L) (3) and an enzymic method (10–47 μmol/L) (4).

Discussion

The Du Pont discrete analyzer system, although convenient, requires a relatively large volume of serum, 250 μL. The manual procedure for the measurement of ammonia is tedious, time consuming, and requires 250 μL of plasma and more than 30 min for completion; moreover, the working reagents and standards have to be prepared freshly for every assay. Inadequate washing of the resin presents problems, and falsely increased ammonia values are easy to obtain.

In contrast, the performance of the ammonia analysis with the dry reagent slide on the Ektachem analyzer offers all the advantages of a “stat” procedure. The analyzer is always ready, calibrated, and programmed to accept the ammonia analysis. Only 10 μL of plasma sample is used and the results can be reported within 5 min, which is ideal for the diagnosis of certain metabolic disorders and Reye’s syndrome in children. This approach, therefore, makes highly reliable and rapid results for ammonia easily available to clinical laboratories serving newborn and acute-care pediatric patients.

References


CLIN. CHEM. 31/12, 2014–2018 (1985)

Evaluation of Dual-Label Simultaneous Assays for Lutropin and Folllotropin in Serum

Cathy J. Beinlich,1 James A. Piper,1 Julia C. O’Neal,1 and Oksan Duruman White2

We evaluated the analytical performance and clinical utility of three dual-label simultaneous assays for lutropin and follitropin by comparison with widely used individual assays for these analytes (Diagnostic Products Corp.; DPC). Of the three assays evaluated, “Cotropin” (Clinites Corp.) and “Combostat” (Micromedics Systems, Inc.) compared favorably with the DPC assay with respect to recovery, linearity, intra- and inter-assay precision, and sensitivity for lutropin. The third assay evaluated, “Simultropin” (Becton Dickinson Immunodiagnostics), exhibited greater inter assay variability for lutropin than did the DPC assay but otherwise compared favorably. Analytically the three assays were similar to one another for follitropin determinations and results compared favorably with those by the DPC assay. Inter correlation of patients’ results obtained with these assays is poor. However, using the same standards with each of the three assays improved intercorrelation of patients’ data except for follitropin by Combostat. A universally accepted reference material for immunoassay of lutropin and follitropin is needed.

Additional Keyphrases: radioimmunoassay · “kit” methods · hormones

Pathological abnormalities in the reproductive system of men and women often require assessment of follitropin (human follicle-stimulating hormone, FSH) and lutropin (human luteinizing hormone, LH) concentration in serum or urine. These analytes usually are determined by radioimmunoassay, and several manufacturers produce kits for such use. However, differences in calibration and specificity of the various assay systems used have generated confusion. Moreover, reference materials for follitropin and lutropin include preparations of both urinary and pituitary origin, which vary in the degree of heterogeneity of molecular

1 Department of Pathology, Medical College of Virginia, Box 597, MCV Station, Richmond, VA 23298.
2 Endocrine Metabolic Institute, 5855 Bremo Rd., Suite 604, Richmond, VA 23226.

Received June 19, 1985; accepted August 30, 1985.
forms of the hormones (1, 2). The urinary preparations include the 2nd IRP-HMG (Second International Reference Preparation of Human Menopausal Gonadotrophins), no longer available, and the 1st IS-HMG (First International Standard of Human Menopausal Gonadotropins), which replaced the 2nd IRP-HMG in 1976. The pituitary preparations include the 1st IRP-FSH/LH (69/104) and the 2nd IRP-FSH/LH (78/549) as well as the 1st IRP-LH (68/40) (3, 4). The potencies assigned to these reference materials are the result of collaborative efforts in 10 to 20 expert laboratories around the world (5).

Recently, several manufacturers have produced kits for the simultaneous radioimmunoassay of lutropin and follitropin in serum. These radioimmunoassays involve the use of $^{67}$Co-labeled lutropin, $^{125}$I-labeled follitropin, and specific antisera for lutropin and follitropin.

Here we report our study evaluating the analytical performance of the dual-label simultaneous assays and comparing the clinical utility of these kits with that of generally accepted products for separate assays for lutropin and follitropin (6). We also determined the differences in values for lutropin and follitropin that result from differences in calibration of the commercial kits.

Materials and Methods

We evaluated the following dual-label procedures for simultaneous assay of lutropin and follitropin, using the kits according to manufacturers' directions: "Cotropin" (Clinetics Corp., Tustin, CA); "Simultropin" (Becton Dickinson Immunodiagnostics, Orangeburg, NY); and "Combostat" (Micromedics Systems Inc., Horsham, PA). The comparison assays for follitropin and lutropin were performed by Endocrine Metabolic Institute, Richmond, VA, using individual assay products from Diagnostic Products Corp. (DPC), Los Angeles, CA. Radioactivity was measured with a Model 1270 "Rackgamma II" gamma counter (LKB Instruments, Gaithersburg, MD). Spillover between the $^{125}$I and $^{67}$Co channels was less than 3%. For data reduction we used a cubic spline-fitting function of counts/min vs log concentration.

LER-907 reference preparation and immunochemical-grade lutropin and follitropin were obtained from the National Pituitary Agency (University of Maryland School of Medicine), National Institute of Arthritis, Metabolism and Digestive Diseases. The immunopotency of LER-907, a partly purified extract of human pituitary glands is expressed in terms of the 2nd IRP HMG (7). LER-907 and the immunochemical-grade materials were diluted in phosphate-buffered saline (per liter, 0.15 mol of phosphate, 0.15 mol of NaCl; pH 7.2) containing 3 g of bovine serum albumin per liter.

Results and Discussion

Table 1 lists the characteristics of the various kits, including the materials used by the manufacturers in calibrating their assay kits. For both Cotropin and Combostat the assay time for lutropin/follitropin is half that for assaysing the two hormones individually. Typically about 75% of requests are for both lutropin and follitropin. We performed assays with different lots of each kit over about three months. Maximum binding ($B_o/T$) was relatively constant from assay to assay except for that for lutropin as measured with the Cotropin kit, which ranged from 7% to 35%. In our first studies the maximum binding was 7% to 19% in lot numbers of the Cotropin kit, but presumably this apparent stability problem for lutropin has been solved: the $B_o/T$ for our last 10 assays varied from 23 to 34% (three lot numbers). The five lots used in this evaluation differed only for the follitropin and lutropin tracers.

Analytical Variables

Figure 1 shows typical dose-response curves for lutropin and follitropin. The Combostat assay showed the greatest change in counts/min over the range of standards considered for lutropin (Figure 1A), whereas the curve for Simultropin is comparatively flattened. For follitropin, the greatest change in counts/min over the range of standards again is given by the Combostat test, the flattest curve being that for the DPC assay.

Reference preparation LER-907 was used to establish the accuracy and analytical recovery of the products investigated, each calibrated with its own calibrators. Several dilutions of LER-907 were assayed and the results for lutropin and follitropin were compared with the calculated values (Table 2). The accuracy for lutropin as measured with the DPC assay was good; however, Combostat consistently overestimated the lutropin concentration. Neither Cotropin nor Simultropin gave the expected results for lutropin. However, both assays did result in a consistent recovery of the reference preparation. The DPC assay most accurately measured follitropin in the reference preparation, although measured values for follitropin exceeded calculated values. Follitropin concentrations as measured with the Combostat kit were 32–35% greater than the calculated values, except at low concentrations, where the overestimation of follitropin was even greater. Analytical recovery of follitropin in both the Cotropin and Simultropin methods increased with decreasing concentration.

For linearity studies we used immunochemical-grade

<table>
<thead>
<tr>
<th>Parameter*</th>
<th>Cotropin</th>
<th>Combostat</th>
<th>DPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH</td>
<td>1st IRP 68/40</td>
<td>2nd IRP 78/549</td>
<td>2nd IRP-HMG</td>
</tr>
<tr>
<td>FSH</td>
<td>WHO 71/333</td>
<td>2nd IRP 78/549</td>
<td>2nd IRP-HMG</td>
</tr>
<tr>
<td>Assay range, int. units/L</td>
<td>0–100</td>
<td>0–100</td>
<td>0–150</td>
</tr>
<tr>
<td>LH</td>
<td>0–150</td>
<td>0–200</td>
<td></td>
</tr>
<tr>
<td>FSH</td>
<td>0–100</td>
<td>2.5 LH, 5 FSH</td>
<td></td>
</tr>
<tr>
<td>Approx. assay time, h</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Sample vol, µL</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Typical $B_o/T$, %</td>
<td>27–38</td>
<td>18–28</td>
<td>29–35</td>
</tr>
<tr>
<td>LFSH</td>
<td>19–32</td>
<td>22–39</td>
<td>27–30</td>
</tr>
</tbody>
</table>

*From manufacturers' inserts except for typical maximum binding. b Individual kits give standard values in terms of both calibrators.
follitropin and pooled patients' sera. All three of the dual-label simultaneous assay kits showed good linearity of dilution curves for follitropin with these two materials (data not shown). Since LER-907 contains 5.6 times more lutein than follitropin, the nonlinear response for follitropin with LER 907 may represent cross reactivity that is not seen when the two analytes are present at similar concentrations.

Intra-assay precision was determined by assaying replicates of pooled patients' sera. The intra-assay precision for lutein and follitropin determined with the dual-label assays was as good as or better than the intra-assay precision of the DPC product (Table 3). The interassay precision was determined by including commercial control material in each assay. The interassay precision for Cotropin was similar to that obtained with the individual assay kits from DPC (Table 3). Both Simultropin and Combiostat had acceptable between-run precision for follitropin but greater than expected precision for lutein. The decreased precision for lutein in the Simultropin assay may be due to the shallow dose–response curve.

Analytical sensitivity was determined by replicate analysis of the zero standard (mean ± 2 SD). The sensitivity for lutein, in int. units/L, was 3.4 (Combiostat), 2.9 (Simultropin), 4.4 (Cotropin), and 2.0 (DPC). The sensitivity for follitropin (int. units/L) was 3.6 (Combiostat), 1.5 (Simultropin), 3.4 (Cotropin), and 1.7 (DPC).

Intercomparison of Kit Results

Correlation studies with patients' samples were done to compare results of dual-label assay with that of the DPC assay (Figure 2). Combiostat and DPC, calibrated in terms of the 2nd IRP-HMG, gave similar values for follitropin. However, the lutein values with Combiostat were about 30% higher than those obtained with DPC (Figure 2A and B). Cotropin, calibrated with the 2nd IRP-FSH/LH 78/549, gave lutein and follitropin values that were 15% lower than those observed with the DPC assays (Figure 2C and D). Simultropin, calibrated with the 1st IRP 68/40 for lutein, resulted in lutein values about 35% lower than DPC values (Figure 2E), and follitropin values obtained with Simultropin was about 60% lower than follitropin measured with DPC (Figure 2F).

The three dual-label simultaneous assays gave discrepant values for lutropin and follitropin in patients' serum. Because differences in calibration could contribute to the observed differences, we also established a standard curve for each assay by using the standards from the Cotropin kit, in addition to the standards provided by the manufacturer. With use of the individual standards provided by the manufacturer, lutropin values obtained with Combiostat were about 60% higher than those obtained with Cotropin. Use of the common standards to calculate patients' values for lutropin resulted in good agreement between results by these two products (Figure 3A). Using the individual standards provided by each manufacturer to compare patients' results for lutropin gave Simultropin = (0.73 Cotropin) – 2.16 (r = 0.91), whereas calculation of patients' results for lutropin by using the standard curve generated with the common standards improved the agreement of patients'
The results of this study indicate that dual-label simultaneous assays for serum follitropin and lutropin can perform analytically as well as individual assays for these analytes.

Of the three products considered, the Simultropin assay is inferior in terms of precision for lutropin, a reflection of the shallow dose-response curve. The results also indicate the importance of establishing assay-specific reference ranges because patients' results vary considerably when measured with different assay products. The major factor contributing to discrepant results for lutropin and follitropin is the use of a variety of calibration materials. Differences in calibration of assay products mean the physician faces a dilemma when attempting to utilize data obtained from different laboratories. This emphasizes the need for a universally accepted calibrator for lutropin and follitropin.

We acknowledge Micromedix Systems, Inc., Clinetics Corp., and Becton Dickinson Immunodiagnostics for providing some of the kits for this evaluation.

References