capacity of the HPLC system with subsequent detection at the relatively low potential of +600 mV are quite selective for catecholamine measurement.

References

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Six Direct Radioimmunoassays of Estradiol Evaluated
Vibeke Schleiter and Jørgen Thode

We evaluated the analytical performance of six commercial direct radioimmunoassays of 17β-estradiol, those from Radioassay Systems Laboratories, IRE Medgenix, Biotecx Laboratories, Farmos Spectria, International CIS, and Diagnostic Products Corp. The mean value for estradiol (E2) and the within- and between-run CVs were determined for serum pools and control materials, measured in seven to 10 runs with each method. Mean values for E2 in pooled sera deviated by 75% to 350% from the means measured with our routinely used extraction method. Between-run CVs ranged from 4% to 14% for the direct assays as compared with 10% for the extraction assay. We also investigated, for two of the direct assays, the effect of extraction with diethyl ether before radioimmunoassay, with respect to improvement in the agreement with our extraction method. All of the assays were easy to perform and results were obtained within 4–5 h, but we conclude that matrix effects may be expected in direct assays of estradiol.

Additional Keyphrases: ovulation induction • matrix effects • monitoring therapy • fertility evaluation • pregnancy

Materials and Methods

Materials
Estradiol radioimmunoassays. Our routinely used E2 assay is the Extraction Estradiol (125I) Radioimmunoassay (Farmos Diagnostica, SF-90460 Oulunsalo, Finland; here abbreviated "Farmos extr."). The direct radioimmunoassays we evaluated were: (a) double-antibody direct (125I) Estradiol-17β (Radioassay Systems Labs., Inc., Carson, CA 90746; "RSL"), (b) OE2-RIA-D (IRE-Medgenix, Fleurus, Belgium; "MED"), (c) Spectria Direct Estradiol (125I) (Farmos Diagnostica; "Spectria"), (d) Estradiol Direct (Biotecx Labs., Inc., Friendswood, TX; "Biotecx"), (e) Estradiol Direct (CIS, F91190 Gif-sur-Yvette, France; "CIS"), and (f) Estradiol Double Antibody (Diagnostic Products Corp., Los Angeles, CA 90045; "DPC").

All assays except Farmos extr. and RSL involve human serum standards with added E2; Farmos extr. and RSL standards are based on bovine serum. 125I-labeled E2 was used as tracer in all seven assays. No additional information about the labeled derivative was obtained from the manufacturers.

The specificity of the antiserum was well documented for all the assays. For the Spectria and the RSL antisera a 20% cross reaction with estrone was reported; for the Biotecx, 4.2%. All seven methods had a low cross reactivity with estriol (<1.5%).

Diethyl ether, analytical grade (E. Merck, Darmstadt, F.R.G.), was used for extraction.

Serum samples. Using each method, we measured two series each of six pooled serum samples, with E2 concentrations ranging from 0.04 to 2 nmol/L.

Control materials. Three control pools (K1, K2, and K3) were prepared from our in-house laboratory control material (5) by addition of increasing volumes of an ethanolic solution of estradiol. Two other control pools were prepared from lyophilized Hormone Controls A (HKA) and B (HKB) from the German Hormone Survey (Deutsche Gesellschaft für Klinische Chemie, D-6500, Bonn, F.R.G.); their E2 content had been measured by mass spectrometry.

Methods

For all the radioimmunoassays we followed the instructions from the manufacturer. The ether extraction proce-
Table 1. Performance of the Assays

<table>
<thead>
<tr>
<th>Method</th>
<th>Sample vol, µL</th>
<th>Detection limit, nmol/L</th>
<th>Upper limit of measuring range, nmol/L</th>
<th>Assay time, h</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farmos extr.</td>
<td>200</td>
<td>0.05</td>
<td>5.5</td>
<td>3</td>
<td>+ extraction</td>
</tr>
<tr>
<td>RSL</td>
<td>50</td>
<td>not stated</td>
<td>11.0</td>
<td>2.5</td>
<td>Special test tubes required</td>
</tr>
<tr>
<td>MED</td>
<td>50</td>
<td>0.02</td>
<td>3.7</td>
<td>4</td>
<td>Coated tubes required</td>
</tr>
<tr>
<td>Spectria</td>
<td>100</td>
<td>0.04</td>
<td>15.0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Biotecx</td>
<td>200</td>
<td>0.04</td>
<td>11.0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>CIS</td>
<td>50</td>
<td>0.02</td>
<td>3.7</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>DPC</td>
<td>200</td>
<td>0.005</td>
<td>1.8</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

*As stated by the manufacturer. aIncubation time + 1 h for preparation of and calculation of results for 10–20 samples; add 1 h for the extraction step, when used.

The incubation time for the assays ranged from 1–3 h; results were obtained within 2–4 h.

Technical details of the assays are listed in Table 1. Serum pools (1–6 or 7–12) and controls (K1, K2, and K3) were measured in seven to 11 runs, in duplicate, with each method except Spectria, with which only three runs were performed. In addition, we measured E2 in lyophilized controls (HKA and HKB) with the Farmos extr., Spectria, Biotecx, CIS, and DPC methods.

Mean (±SD) concentrations of E2, in nmol/L, as measured with the Farmos extr. method were: (pool 1–6): 0.08 ± 0.10, 0.24 ± 0.04, 0.43 ± 0.04, 0.82 ± 0.10, 1.13 ± 0.12, 1.99 ± 0.25; (pool 7–12): 0.02 ± 0.026, 0.07 ± 0.026, 0.17 ± 0.03, 0.32 ± 0.05, 0.51 ± 0.06, 0.81 ± 0.11; (K1–K3): 0.14 ± 0.02, 0.97 ± 0.10, 2.02 ± 0.17; (HKA, HKB): 0.80 ± 0.04, 1.66 ±
0.09. The values by mass spectrometry for HKA and HKB were 0.80 and 1.56 nmol/L.

We used the "Gamma Master" gamma counter (LKB Wallac, Turku, Finland) to measure the radioactivity. This instrument also calculated the results, by third-order spline functions for standard curve fitting.

We used Student's t-test with the Statgraphics Statistical Program (version 2.1) to test the significance of deviations of means. To calculate precision profiles we used cumulated deviations between duplicates, as described by Ekins (6).

**Results**

Figure 1 shows the relationship (expressed as ratios) between values for E₂ as measured by the direct methods and those measured by the extraction method. Compared with the extraction method, RSL, Spectria, and Biotecx overestimated the concentrations of E₂ in the serum pools (RSL by 1.5 to 3 times, Spectria by 2 to 6 times, and Biotecx by 2.7 to 25 times), the discrepancies being highest for low values of E₂ and decreasing towards higher E₂ values. The differences were less for frozen controls, and even less (although still significant) for lyophilized controls. MED overestimated low values of E₂ and underestimated high values at approximately the same rate for frozen controls as for serum pools. CIS agreed well with the extraction method for serum pools, but not for frozen and lyophilized controls, the latter being underestimated by 40%. The DPC assay showed only small deviations for serum pools and frozen controls, as compared with the extraction method, although overestimation was significant for some of the serum pools; even so, the concentration of E₂ in frozen and lyophilized controls was underestimated by the DPC assay. Results by the Farmos extr. agreed well with the values by mass spectrometry for HKA and HKB (ratios 1.00 and 1.06, respectively).

As observed (Figure 1), extraction of samples and calibration standards with ether before assay decreased the degrees of overestimation considerably for Spectria and, especially, for Biotecx.

Figure 2 shows within-run and between-run precision profiles for the evaluated assays. Within-run and between-run CVs were less than 10% for Spectria, DPC, RSL, and Biotecx for the whole measuring range; however, for Biotecx a constant bias was found for values below 0.5 nmol/L. We found higher CVs for MED and CIS above 1 nmol/L and high between-run variation for MED in the whole measuring range.

**Discussion**

We chose the Farmos extr. method as our comparison method because it has been our routine method for several years and results agree well with mass-spectrometric values of the German Quality Control Scheme. Our reference schemes are based on that method.

The precision for most of the six assays of E₂ we evaluated was acceptable for clinical use. With the exception of one assay, the precision was better than that of the extraction assay—as we expected, owing to the simplification of the analytical procedure. However, the large and variable differences in the measured concentrations of E₂ between methods for pooled sera and control materials cannot be explained only by differences in the calibration and the antibody cross-reactions, but also include a matrix effect, as supported by the fact that an extraction procedure done before assay (Spectria and Biotecx) decreased the differences between results for E₂ for pooled sera as well as for controls.

We did not use regression analysis for the method comparisons, because the extraction method cannot be regarded as an error-free independent variable and because the results differed for pooled sera and controls (7, 8).

The use of both pooled sera and different control materials in our investigation showed that, with direct assays of E₂, results for control materials, frozen or lyophilized, are not comparable and therefore do not necessarily reflect the differences that may be expected when patients' samples are analyzed. The variable interference found in our study may explain the disagreement of correlations calculated from patients' samples and correlations calculated from control material found by Nisbet and Jomain (9). Diver (10) also
found large differences in results of E₂ in plasma from neonates with direct assays of E₂ with and without extraction of samples.

The difficulties in direct measurement of E₂ in serum may be traced to the fact that only 2% of this hormone circulates as free hormone, the major part being strongly bound to sex-hormone-binding globulin and, more weakly, to albumin (11), combined with the use of an ¹²⁵I-labeled estradiol derivative as assay tracer. None of the assays included in the present evaluation are supplied with information as to which ¹²⁵I-labeled estradiol derivative is used, and only one supplier states that an inhibitor for blocking the binding globulin is included in the tracer.

The choice of method depends on the clinical application (e.g., the need for low detection limit or for a broad measuring range (8, 9). The RSL, Spectra, and Biotecx, which have very broad measuring ranges, may be used for monitoring ovulation induction therapy. In our laboratory, measurements of E₂ are requested for endocrinological evaluation of men and of menopausal and infertile women as well as for monitoring ovulation induction therapy and early pregnancy. The DPC assay was chosen in our laboratory because of sufficient agreement between results of E₂ with the extraction method, low detection limit, and good precision. We use a sample-dilution step when the concentration of E₂ exceeds 2 nmol/L.

We conclude that the selection of a direct radioimmunoassay of E₂ cannot be based only on the correlation of the assay results with gas-chromatography/mass-spectrometry values for a lyophilized control material, because differences between direct methods and an extraction method are often much larger for patients' sera than for lyophilized controls. The matrix effect that we observed in this study warrants further investigations.

Reagents were supplied in part by the Danish suppliers: Farmos Diagnostics, Apodan, Rick, Novo Biotecx, Interkemi, and Kingo Diagnostics.

References

Lack of Effect of Warfarin on Uric Acid Concentration
Frederic B. Walker, IV, Daniel M. Beckar, Beth Kowal-Neesley, and Linde S. Krongaard

Uric acid concentrations reportedly are increased in patients being treated with warfarin. We measured uric acid in 40 patients before and during warfarin administration. The mean pre- and post-warfarin uric acid concentrations for our patients were 0.39 mmol/L and 0.40 mmol/L, respectively, not a significant difference. Further observations of hyperuricemic patients started on warfarin are needed to prove that their risk of gout is not increased.

Menon et al. (1) recently reported that therapy with warfarin increases uric acid concentrations in plasma. Because this association has important theoretical implications for patients with hyperuricemia and (or) gout who are being treated with warfarin, we attempted to reproduce their findings.

Methods
Because of the many clinical variables that can affect uric acid concentration, such as medications, changes in renal function, and genetic predisposition, we decided to let each patient serve as his/her own control in a paired analysis. From our anticoagulation clinic we selected 40 patients for whom uric acid had been determined before warfarin therapy. If patients had more than one pre-warfarin uric acid value, we used the measurement made nearest to the onset of anticoagulation. We measured serum uric acid again after the patients were started on warfarin, and we recorded duration of therapy and dose of warfarin for all patients. Warfarin dose was adjusted to maintain the prothrombin time to about 1.5 times the control value.

Serum uric acid was measured by an enzymatic uricase method, in a smac AutoAnalyzer (Technicon Instruments, Tarrytown, NY). Our normal reference interval is 0.15 to 0.48 mmol/L for men and 0.09 to 0.42 mmol/L for women (mean ± SD for a group of normal individuals). We compared uric acid values before and during warfarin therapy by a paired t-test and the Wilcoxon signed-rank test.