Evaluation of a New Commercial Solid-Phase Direct Radioimmunoassay for Unconjugated Estriol in Pregnancy Plasma

J. T. France, B. S. Knox, and P. R. Fisher

We evaluated a new radioimmunoassay kit for unconjugated estriol in pregnancy plasma. The overall mean intra-assay precision (CV), as determined from replicate analyses of three plasma pools with different estriol concentrations, was 5%; the overall mean inter-assay precision was 7.7%. The assay system had acceptable linearity, with a correlation coefficient of 0.97 between results for 24 plasma samples assayed at 10 and 20 μL. Analytical recovery of estriol added to plasma to give three concentrations was generally higher with the kit than with our conventional charcoal-separation RIA method. The regression line equation was y = \(1.11x + 1.0\), the correlation coefficient 0.97. In plasma from 28 normal pregnant women, sampled serially during the last trimester, the mean unconjugated estriol concentration in plasma increased steadily from 29 nmol/L at 28 weeks of gestation to 42 nmol/L at 34 weeks, and then more rapidly to 93 nmol/L at term. This kit provides a rapid, technically simple, and reliable assay method, offering advantages to clinical laboratories with a high estriol workload.

Since the early 1960s, measurement of the maternal 24-h urinary excretion of estriol has been used as a laboratory test of fetoplacental function (1), though more recently, with the development of radioimmunoassay (RIA), determination of the concentration in the blood, either of unconjugated estriol or total estriol, has offered an alternative approach (2). Apart from certain unusual conditions of pregnancy, the 24-h urinary estriol, the plasma unconjugated estriol concentration, and the plasma total estriol concentration all appear to provide information of comparable clinical significance.

Plasma (or serum) assays have an advantage over 24-h urine assays in that sample collection is more convenient, but on the other hand there is a diurnal variation in plasma estriol concentration (3). There is disagreement over which analytical approach is superior, but in practice the choice in a clinical laboratory service often depends on the demands placed on the service. Where there is a large workload, urinary estriol is generally favored because continuous-flow methods such as those of Brombacher et al. (4) and Lever et al. (5) enable fast assay of many samples at low cost. Where the workload is relatively small, making the use of an AutoAnalyzer or some equivalent instrument impractical, plasma estriol measurements by RIA, which may offer a shorter assay time and usually better precision, are preferred to manual or semi-automated assays for urinary estriol.

The Radiochemical Centre, Amersham, England, recently introduced a new RIA assay system for determining unconjugated estriol in pregnancy plasma. The Amersham "Amerlex" assay has both the simplicity of procedure and the speed required for rapid, same-day analysis of many samples.

Potentially, the Amerlex assay now makes plasma estriol determinations a practical alternative to urinary estriol measurements in a high-workload laboratory service. This paper reports our evaluation of the Amerham Amerlex assay system and the ranges we found for plasma unconjugated estriol during the last 13 weeks of normal pregnancy as determined with this assay.

Materials and Methods

For pipetting steps we used Eppendorf or SMI pipettors. The Amerlex estriol (unconjugated) RIA kit evaluated in this study was obtained from The Radiochemical Centre, Amersham, U.K., through Amersham Australia Pty Ltd.

The assay is a direct solid-phase RIA method involving a specific antisemurn having low cross reactivity with estriol conjugates. Estriol-3-glucuronide has a 3.9% cross reaction and estriol-3-sulfate a 2.1% cross reaction with the antisemum (6). Estetrol (30.7%) and 16-epiestriol (9.0%) also have significant cross reactivities, but the circulating concentrations of these estrogens are much lower than for estriol. In outline, the assay procedure is as follows:

In duplicate, place 20-μL aliquots of the plasma or serum samples into 75 × 10 mm (i.d.) polystyrene test tubes and add 100 μL of 125I-labeled estriol solution, followed by 500 μL of "Amerlex" bound estriol-antibody suspension. Vortex-mix and leave the tubes at room temperature for 30 min. Then centrifuge in decanting racks at 1500 × g for 15 min, invert the racks of tubes to remove the supernatant fluid, and invert them on a pad of cotton wool to drain for 5 min. Then place the tubes in a gamma counter (we used a Searle Analytic Inc. Model 1185) to measure the amount of 125I-labeled estriol bound to antibody in the pellet. Prepare a six-point dose-response standard curve covering the concentration range from 0 to 174 nmol of estriol per liter with each assay batch, using standard serum preparations provided with the assay reagents.

For the present study, the curve as a plot of percent 125I-labeled estriol bound vs estriol concentration was hand drawn with the aid of a SICI mathematical curve (Faber Castell, Germany). A typical standard curve is illustrated in Figure 1. Nonspecific binding for the assay averaged 2.8 (SD 0.7)% of the total counts.

Results

Precision and Linearity of Assay

The within-assay and between-assay precision of the method was determined from repeated analyses of three plasma pools with different estriol concentrations. At an estriol concentration of 22.2 nmol/L, the within-assay precision (CV) was 4.6% (n = 20) and the between-assay precision was 5.7% (n = 15). At an estriol concentration of 33.3 nmol/L, these values were, respectively, 3.8% (n = 20) and 9.7% (n = 15), and at a concentration of 75.3 nmol/L, they were 6.5% (n = 20) and 7.8 (n = 15). Overall, the mean CV within assay was 5.0% and 7.7% between assay.

To investigate the linearity of the assay system, we assayed 24 different plasma samples, using a 10-μL plasma sample (+
10 μL buffer) and a 20-μL of plasma sample. The analyses were done in duplicate. The estriol concentration in the plasma samples ranged from 43.7 to 165 nmol/L. When the results obtained with the 10-μL sample (y-axis) were correlated with the results from the 20-μL sample (x-axis), the regression, which was linear, was described by the equation \( y = 0.89x + 2.9 \) and the correlation coefficient was 0.97.

**Analytical Recovery of Added Estriol**

Estriol was added to a pool of human male plasma to produce a concentration of 86.8 nmol/L. A sample of the plasma was then serially diluted with more of the plasma pool to give concentrations of 57.8 and 28.9 nmol/L. The three plasmas were then assayed (in replicates of 20) to determine the accuracy of the method as reflected in the exogenous estriol accounted for. The results are listed in Table 1. For the 28.9 nmol/L concentration, the recovery was 99.0%; for 57.8 nmol/L, 100.7%; and for 86.8 nmol/L, 96.2%.

The concentration of unconjugated estriol in plasma from normal men is below the sensitivity of the assay. The value of 2.2 nmol/L obtained for the male plasma pool in the above experiment (Table 2) may therefore be regarded as indicating the value for the assay blank.

**Comparison with Charcoal-Separation RIA Method**

The Amerlex assay was compared with our own in-house plasma unconjugated estriol RIA (7), for which the protocol involves extracting the plasma with diethyl ether and separation of free and bound fractions on dextran-coated charcoal. We assayed 15 pregnancy plasma samples in duplicate by both methods. With the Amerlex assay, the individual plasma results ranged from 21.2 to 106 nmol/L (mean, 15.8 nmol/L). With the charcoal separation method, the values ranged from 22 to 92 nmol/L (mean, 14.5 nmol/L). Overall, the Amerlex assay gave slightly higher but significantly different values (\( p < 0.01 \), paired \( t \)-test). The regression line (y = Amerlex result) was described by the equation \( y = 1.11x + 1.0 \); the correlation coefficient was 0.97.

**Normal Values in Pregnancy**

We measured unconjugated estriol in plasma by the Amerlex method serially from 28 weeks of gestation until term in 28 normal pregnant women, a total of 192 samples. Samples from each individual patient were included in the same assay batch. Our criteria for normal pregnancy were as follows: (a) a known date for the last menstrual period; (b) an absence of complications of pregnancy; (c) the pregnancy ending within a gestational period of 40 weeks ± 10 days with spontaneous onset of labor leading to vaginal delivery of a live baby whose weight exceeded 2800 g.

We calculated mean weekly (± three-day range) plasma concentrations of estriol from results for the assayed samples. Concentrations increased steadily from 29 nmol/L at 28 weeks to 42 nmol/L at 34 weeks, then more rapidly to reach a mean of 93 nmol/L at 40 weeks of gestation. Table 2 lists the mean values with 95% confidence limits (obtained after logarithmic transformation to normalize the data). These results are shown graphically (after smoothing of values) in Figure 2.

**Discussion**

We find this kit to be a simple procedure, involving three pipetting steps, a centrifugation, and measurement of bound labeled estriol in a gamma counter. The assay system offers the following advantages:

- being a direct RIA, ether extraction is obviated

**Table 1. Analytical Recovery of Authentic Estriol Added to Human Male Plasma, as Determined by the "Amerlex" Assay System**

<table>
<thead>
<tr>
<th>Added Estriol, nmol/L</th>
<th>Found, nmol/L</th>
<th>Found of added %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.2 ± 0.97 (n = 6)</td>
<td>—</td>
</tr>
<tr>
<td>28.9</td>
<td>30.8 ± 2.3 (n = 20)</td>
<td>99.0</td>
</tr>
<tr>
<td>57.8</td>
<td>60.4 ± 5.1 (n = 20)</td>
<td>100.7</td>
</tr>
<tr>
<td>86.8</td>
<td>85.7 ± 6.8 (n = 20)</td>
<td>96.2</td>
</tr>
</tbody>
</table>

![Image](image_url) Fig. 1. Typical dose–response standard curve obtained with the kit.

![Image](image_url) Fig. 2. Mean 95% confidence limits (smoothed) for unconjugated estriol in plasma during the third trimester of pregnancy, as determined from serial measurements in 28 normal pregnant women.
Table 2. Mean and 95% Confidence Range for Unconjugated Estriol in Plasma from 28 Normal Pregnant Women during the Last 13 Weeks of Gestation

<table>
<thead>
<tr>
<th>Weeks of gestation</th>
<th>28</th>
<th>29</th>
<th>30</th>
<th>31</th>
<th>32</th>
<th>33</th>
<th>34</th>
<th>35</th>
<th>36</th>
<th>37</th>
<th>38</th>
<th>39</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unconjugated estriol, nmol/L</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper limit*</td>
<td>39.9</td>
<td>47.5</td>
<td>46.2</td>
<td>62.8</td>
<td>58.0</td>
<td>68.7</td>
<td>66.6</td>
<td>97.9</td>
<td>106.0</td>
<td>123.0</td>
<td>144.0</td>
<td>157.0</td>
<td>160.0</td>
</tr>
<tr>
<td>Mean</td>
<td>29.2</td>
<td>30.2</td>
<td>30.9</td>
<td>34.4</td>
<td>39.6</td>
<td>37.8</td>
<td>41.6</td>
<td>53.4</td>
<td>64.9</td>
<td>70.1</td>
<td>80.9</td>
<td>88.5</td>
<td>93.3</td>
</tr>
<tr>
<td>Lower limit</td>
<td>20.5</td>
<td>18.4</td>
<td>19.8</td>
<td>17.0</td>
<td>25.7</td>
<td>19.1</td>
<td>24.6</td>
<td>26.7</td>
<td>37.5</td>
<td>41.3</td>
<td>45.5</td>
<td>50.3</td>
<td></td>
</tr>
<tr>
<td>No. samples</td>
<td>10</td>
<td>8</td>
<td>12</td>
<td>8</td>
<td>13</td>
<td>12</td>
<td>13</td>
<td>18</td>
<td>16</td>
<td>24</td>
<td>20</td>
<td>25</td>
<td>13</td>
</tr>
</tbody>
</table>

* 95% confidence limits represent ± 2SD from the mean, calculated after logarithmic transformation of the data.

- because the estriol antibody is bound to polymer particles, free and bound fractions are readily separated by centrifugation
- incubation is brief: 30 min at room temperature
- 125I-labeled estriol is used as the tracer, resulting in a shorter counting time and avoiding the need for scintillation fluid

With the aid of a programmable calculator (such as a Hewlett Packard 97S) and a curve-fitting program to process the counting data, an assay run of 150 tubes can be completed in a single working day by one technician and results reported the same day.

A very acceptable level of precision was obtained with the assay when the counting data were hand plotted as directed in the assay kit instruction booklet. An improved inter-assay precision might be expected if the standard curve was plotted with the aid of a programmable calculator or computer using a curve-fitting program.

This kit may give higher estriol values than do conventional RIA methods that involve ether extraction and charcoal separation. When directly compared with our in-house estriol RIA, it gave significantly higher results (p <0.01). Furthermore, higher values for unconjugated estriol in plasma were obtained for normal pregnant women than generally reported in other studies (2, 8), including our own (7). The values for normal pregnancy, particularly during the last six weeks of gestation, are also higher than the reference estriol concentrations described in the booklet provided with the assay kit, perhaps reflecting differences in the groups of patients studied. In any case, the findings emphasize the importance of each laboratory's determining its own normal reference range.

The kit, because of its technical simplicity and speed of analysis, for the first time offers to the laboratory with a high demand for estriol assays the possibility of providing plasma unconjugated estriol as a realistic alternative to urinary estriol measured by continuous-flow analysis, although the higher reagent costs could be an important consideration.

We thank Amersham Australia Pty. Limited for donating the kit.

References

6. From data in booklet provided with the Amersham “Amerlex” oestriol (unconjugated) RIA kit, Table 4, p 12.