Turbidimetric Latex Immunoassay of Placental Lactogen on Microtiter Plates

D. Collet-Caesar, J. N. Limet, L. Van Krieken, and R. De Hertogh

In this latex immunoassay for human placental lactogen, microtiter plates are used as the reaction vessel and the absorbance at 405 nm is measured to quantify the reactions. This 30-min assay necessitates only one serum dilution and two pipetting steps. The calibration curve extends from 0.5 to 15 mg/L. CVs range from 4.2% to 7.0% for within-run determination and from 7.0% to 11.2% for between-run determinations. A correlation coefficient of 0.949 was obtained for 84 sera when the method was compared with a commercial radioimmunoassay.

Additional Keyphrase: radioimmunoassay compared

Determination of human placental lactogen (HPL) in maternal serum is useful for assessing placental function (1). Assay methods for HPL have been described, including radioimmunoassay (2-4), enzyme immunoassay (5), fluorimunoassay (6), and particle-counting immunoassay (7). The last-named method, based on the agglutination of antibody-coated latex particles, represents an interesting alternative to radiolabeled techniques: the reagents are non-radioisotopic and the assay is homogeneous and rapid, allowing automation. Basic principles and several applications of this technique have been described elsewhere (8). Despite obvious advantages, implementation of this technique has been slow, owing to limited availability of particle-counting systems.

To exploit the intrinsic advantages of latex-particle immunoassays and to overcome the problem of availability of counters, we have developed a latex immunoassay for HPL on microtiter plates, using a microtiter plate reader to quantify latex agglutination. We present here some characteristics of the assay and its comparison with a commercial radioimmunoassay (RIA).

Materials and Methods

Materials

Glycine-buffered saline (GBS): per liter, 0.17 mol of NaCl, 0.1 mol of glycine, and 40 mg of sodium azide; pH adjusted to 9.2 with 8 mol/L NaOH.

GBS-HSA: GBS buffer containing human serum albumin (Behringwerke, Marburg, F.R.G.), 1 g/L.

GBS-BSA-T: GBS buffer containing 10 g of bovine serum albumin (Fraction V, United States Biochemical Corp., Cleveland, OH) and 10 mL of Tween 20 surfactant (Technicon Instruments Corp., Tarrytown, NY 10591) per liter.

HPL standards: HPL standard serum from Behringwerke containing HPL, 8700 μg/L, diluted in GBS-BSA-T buffer and used to calibrate the reaction system.

Samples: Blood samples were centrifuged for 10 min at 4000 × g and the serum was collected and stored at 4 °C or frozen until use.

Equipment: A Titertek vortexing tray (Flow Laboratories, Herts, U.K.) was used as shaker for flat-bottomed microtiter-plates (Greiser, Nürtingen, F.R.G.). Turbidimetric measurements at 405 nm were performed with a Titertek Twin Reader.

Methods

F(ab)2 fragments of anti-HPL: Anti-HPL IgG (Dakopatts, Glostrup, Denmark) was digested with twice-crystallized pepsin (Sigma Chemical Co., St. Louis, MO), 2 mg of pepsin per 100 mg of protein, in acetate buffer (0.1 mol/L, pH 4.5) for 20 h at 37 °C. The digestion was then stopped by adding solid Tris (Merck, Darmstadt, F.R.G.) to increase the pH to 8. The F(ab)2 fragments were then purified by chromatography on Ultrogel AcA44 (LKB, Bromma, Sweden). After concentration and dialysis against isotonic saline, the F(ab)2 fragments were stored at −20 °C.

Latex: Carboxylated latex particles (Estapor K150, 0.8-μm diameter, Rhone-Poulenc, Courbevoie, France) were covalently coated with F(ab)2 fragments as described previously (9) except that we used HSA in the buffer to stop the reaction; the protein/latex weight ratio was 1/50. The coated latex was suspended at a concentration of 2 g/L in GBS-HSA buffer. This suspension is stable for at least one month at 4 °C. For long-term storage, the latex particles can be lyophilized or frozen at −20 °C without losing activity.

Assay procedure: Dilute serum samples 100-fold in GBS-BSA-T buffer, then dispense 50 μL of diluted sera or standards into each well of the microtiter plate. Add 50 μL of latex particles to each well and mix. Measure absorbance immediately to obtain blank values (T0), then cover the plate with plastic tape and agitate for 30 min on the microtiter-plate shaker. Measure the absorbance again (T1). The decrease in absorbance (ΔA = T0 - T1) is directly proportional to the HPL concentration. Perform all measurements in duplicate.

Comparison assay: We also assayed the sera with Behring's "RIA-GNOST HPL," calibrated with the International Reference Preparation (IRP) 73-545 according to the specifications of the manufacturer.

Results

Calibration curve: When ΔA is plotted vs the log of HPL concentration, the resulting calibration curve (Figure 1) shows a sigmoidal shape as in other immunoassays. No reagent is added to stop the reaction before reading results, so it is important to perform optical measurements when the agglutination reaction is at or near equilibrium. We found that after 30 min, the agglutination curve did not change significantly. HPL concentrations in the wells ranged from 2 to 200 μg/L; because the sera are diluted 100-fold before

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3 Nonstandard abbreviations: HPL, human placental lactogen; GBS, glycine-buffered saline; HSA, human serum albumin; GBS-BSA-T, GBS containing bovine serum albumin and Tween 20 (see text).

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assay, the actual concentration range of the assay is between 0.2 and 20 mg/L, with a dynamic range of 0.4 to 12 mg/L. A high-dose “hook” effect has been detected for concentrations exceeding 50 mg/L. This concentration is never seen in serum from pregnant women, so it has no effect on the interpretation of the results. Serum-to-serum variation of absorbance owing to intrinsic color is eliminated by performing blank measurement. In fact, at the given dilution practically no serum samples absorb light at 405 nm; only hemolyzed sera somewhat increase the absorbance of the reaction wells.

Analytical recovery: Three groups of 10 sera each were respectively supplemented with 0.55, 1.15, and 2.90 mg of HPL per liter and assayed (Table 1). Four sera with initial HPL concentrations between 1 and 13 mg/L were serially diluted and the concentrations measured (Figure 2).

Precision: To assess within-run reproducibility, we assayed four sera seven times the same day, using the same standard curves. Between-run reproducibility was estimated by repeating the same assays during 12 days. Results are reported in Table 2.

Correlation: We assayed 84 sera with turbidimetric latex immunoassay (y) and RIA (x). HPL concentrations ranged from 0.4 to 12.9 mg/L. Results of the two methods (Figure 3) were related by the regression line: \( y = 0.978x - 0.45 \) (\( r = 0.949 \)).

**Discussion**

The need for non-radioisotopic immunoassays to replace RIAs led to the development of several technologies, among which enzyme immunoassays performed with coated microtiter plate are the most widely used. More and more companies are proposing equipment such as dilutors, incubators, shakers, readers, and related softwares designed for microtiter plate or strips.

Among other alternatives to non-radioisotopic immunoassays, the latex particle-counting technique seems to have powerful potentials. It is fast, versatile, sensitive, and homogeneous, but the requirement for a dedicated particle counter (8) has limited the users of this technology. Although several turbidimetric and nephelometric assays based on latex agglutination have been developed, they too require expensive automated apparatus (10–13).

The combination of latex agglutination chemistry and commonly used microtiter-plate equipment appears promising as a tool for clinical immunoassays. In the present technique, we exploited what we had learned about immunoassays by latex particle counting (7–9), in particular the use of F(ab')2 fragments of antibodies to avoid interference by rheumatoid factor.

![Fig. 1. Calibration curve for the determination of HPL](image)

![Fig. 2. Dilution curves for four sera by the present method](image)

**Table 1. Analytical Recovery of HPL in the Assay**

<table>
<thead>
<tr>
<th>Added HPL (mg/L)</th>
<th>Recovered, mean (and SD)</th>
<th>Recovery, mean (and SD),%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.55</td>
<td>0.51 (0.06)</td>
<td>92.5 (12.7)</td>
</tr>
<tr>
<td>1.15</td>
<td>1.16 (0.01)</td>
<td>101 (10)</td>
</tr>
<tr>
<td>2.90</td>
<td>2.74 (0.20)</td>
<td>94.5 (7.4)</td>
</tr>
</tbody>
</table>

**Table 2. Precision of the Assay**

<table>
<thead>
<tr>
<th>Mean (and SD), mg/L</th>
<th>CV, %</th>
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<tbody>
<tr>
<td><strong>Within-run</strong></td>
<td></td>
</tr>
<tr>
<td>1.42 (0.06)</td>
<td>4.2</td>
</tr>
<tr>
<td>3.19 (0.21)</td>
<td>6.6</td>
</tr>
<tr>
<td>4.27 (0.30)</td>
<td>7.0</td>
</tr>
<tr>
<td>7.81 (0.34)</td>
<td>4.3</td>
</tr>
<tr>
<td><strong>Between-run</strong></td>
<td></td>
</tr>
<tr>
<td>1.43 (0.16)</td>
<td>11.2</td>
</tr>
<tr>
<td>3.19 (0.22)</td>
<td>8.8</td>
</tr>
<tr>
<td>4.24 (0.44)</td>
<td>10.4</td>
</tr>
<tr>
<td>7.89 (0.55)</td>
<td>7.0</td>
</tr>
</tbody>
</table>

*a Seven assays repeated within the same day with one standard curve.

*b Assays repeated during 12 days with a new standard curve each day.
Although the sensitivity of the assay suffices for HPL determination, preliminary comparisons with particle-counting measurements (J. Limet, personal communication, to be published) show that the latter is more sensitive by a factor of 2 to 40. The turbidimetric latex immunoassay involving microtiter plate equipment that we describe here presents several interesting features and, although it doesn't seem to reach the high sensitivity of particle counting, it could be applicable to a large spectrum of analytes.

We thank Miss Jottile Lambert for excellent technical assistance.

References

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Effect of Ether or Ketamine Anesthesia on Rat Uterine Estrogen and Progesterone Receptors

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Rat uterine estrogen receptors (ER) and progesterone receptors (PR) have been used as controls in ER and PR assays of breast tumors. Stunng or decapitation of experimental animals without prior anesthesia is no longer acceptable as a method of killing. Thus, we compared the effects of two anesthetics on the concentration of rat uterine ER and PR. Rats were killed by one of three methods: (a) stunning, (b) ether anesthesia followed by decapitation, or (c) ketamine anesthesia followed by decapitation. ER and PR concentrations were determined by titration assay, with dextran-coated charcoal separation, and quantified by Scatchard analysis.

No significant differences were found in mean receptor concentrations or dissociation constants for the three groups. The results indicate that there is no residual effect of diethyl ether or ketamine hydrochloride on the binding of either estrogen or progesterin to their respective receptors. The use of decapitation after ether or ketamine anesthesia is appropriate for measuring ER and PR receptors in rat uterus.

Additional Keyphrases: veterinary chemistry • medical ethics

Since Korenman (1) proposed the quantitative assay of uterine estrogen receptor (ER) by the dextran-coated charcoal assay, untold numbers of rats have been used for the collection of uterine receptors for isolation, identification, and mechanism of action studies. In addition, rat uterine ER and progesterone receptor (PR) are being used as inter-assay and intra-assay controls for clinical assays of breast tumor ER and PR (2–4). It is necessary to find a method of euthanasia that is humane and does not affect the quantifi-