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^{125}I-Labeled Radioimmunoassay Kits for Progesterone Evaluated for Use in an In Vitro Fertilization Program

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We have evaluated two commercially available ^{125}I radioimmunoassay kits (Diagnostic Products Corp., DPC; and Radioassay Systems Laboratories, RSL) for measurement of serum or plasma progesterone, to determine their suitability for use in in vitro fertilization programs. Both kits were suitably rapid for program requirements. Results by both were linear with concentration up to 60 nmol/L, and both had acceptable lower detection limits of 0.3 nmol/L. Kit-determined progesterone concentrations (y) for 100 patients' samples correlated well with results by our existing ^{3}H radioimmunoassay method ($y = 1.11x + 0.2$, $r = 0.965$ for the DPC kit; $y = 1.01x + 1.4$, $r = 0.974$ for the RSL kit). Mean analytical recovery for the RSL kit was 116%, that for the DPC kit, 202%. Within-batch precision, expressed as the mean CV for three concentrations of progesterone, was 6.5% for the RSL kit, and 16.4% for the DPC kit; between-day CV was 8.1% for the RSL kit, 17.7% for the DPC kit. We conclude that the RSL kit provides a rapid, precise, and accurate assay for serum progesterone, suitable for use in a fertilization program, but do not recommend the DPC kit for either this purpose or the more general purpose of tracking menstrual cycles.

Additional Keyphrases: *in vitro fertilization · steroids · estrogens · monitoring menstrual cycles*

Progesterone is produced and released by the corpus luteum. A small increase in the concentration of plasma progesterone from the basal follicular value, which is usually very low or undetectable, indicates that luteinization has commenced and that ovulation is imminent; in contrast, a high concentration in plasma confirms that ovulation has occurred. Serum progesterone is generally measured with a tritium label radioimmunoassay (RIA) of a serum extract (*1*); extraction with organic solvents increases the specificity and sensitivity of the measurement. This slow and cumbersome technique has been adequate for clinical applications in tracking and assessing menstrual cycles. However, laboratories are now becoming involved with in vitro fertilization programs, which require the time of the natural surge of lutropin (luteinizing hormone) to be predicted so that an artificial surge of lutropin activity (as human choriogonadotropin, HCG) may be created and the ova harvested as close as possible to the time of natural ovulation. If early luteinization of the follicle has commenced before the HCG is administered, the drug and egg-harvesting protocol may require modification.

Measurement of plasma progesterone, in conjunction with plasma estradiol, currently provides the best available biochemical forecast of follicular development. However, to be of practical use in an in vitro fertilization program, the plasma progesterone assay has to be rapid (<4 h) and precise at low concentrations of steroid (<10 nmol/L). The time requirement can be met by using unextracted samples and ^{125}I as the RIA label. To retain its traditional usefulness for tracking menstrual cycles, such a method must also have acceptable performance characteristics at higher concentrations of progesterone (>20 nmol/L). Several commercial kits that appear to fulfill these criteria have recently become available. We have evaluated two such kits: a solid-phase RIA from Diagnostic Products Corp., Los Angeles, CA 90045 ("Coat-a-Count" No Extraction Progesterone; DPC); and a liquid-phase RIA from Radioassay Systems Laboratories Inc., Carson, CA 90746 (RSL ^{125}I Progesterone kit; RSL). We compared the performance of these kits with that of our well-established in-house [^{3}H]progesterone assay, the analytical performance characteristics of which are acceptable for general diagnostic use.

Materials and Methods

Our evaluation protocol was based on accepted criteria (*2–7*). All assays were performed by the same operator exactly according to manufacturer's instructions. The DPC kit utilizes 100 μL for both standard and sample addition, 1.0 mL for [^{125}I]progesterone, and an incubation period of 3 h at room temperature. The volumes required by the RSL kit are: 100 μL for both standards and samples, 500 μL for anti-progesterone serum, 200 μL for [^{125}I]progesterone, and 100 μL for the second-antibody reagent. The assay tubes are incubated at 37 °C for 1 h, followed by centrifugation at 1000 × g for 15 min. Both kits allowed a choice between decantation and aspiration at the separation step; with both kits we chose to decant. After draining, visible moisture was removed with individual cotton pledgets without disturbing antibody-bound radioactivity. For both ^{125}I kits the antibody-bound radioactivity was determined for 1 min, in a Nuclear Enterprises NE 1600 gamma counter. To ensure that all the antibody-bound radioactivity was consistently available for detection in the counter wells in the case of the solid-phase DPC kit, 1.1-mL aliquots of [^{125}I]progesterone (cpm approximately equivalent to bound radioactivity for the highest standard) were dispensed into 16 solid-phase tubes and the radioactivity was counted for 1 min (mean, 1986 cpm; CV = 2.4%).

For the in-house ^{3}H method the progesterone is extracted with hexane (10 mL) from 500 μL of serum and then taken into aqueous buffer, of which 100-μL aliquots are subjected to conventional RIA, with use of a 1-h incubation step and dextran/charcoal (0.6 and 6 g/L) separation. Antibody-bound radioactivity is determined during 10 min in a Packard Tri-Carb liquid scintillation spectrometer. Progesterone standards are in aqueous buffer, and sample results are corrected for extraction efficiency.

All standards and unknowns are assayed in duplicate and the results calculated from the means of these duplicates. Percentage differences between the individuals of duplicate pairs (C_1, C_2) were calculated as $[(C_1 - C_2)/0.5(C_1 + C_2)] \times 100$.

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Precision. Three pools of human sera containing clinically significant concentrations of progesterone were lyophilized in aliquots and stored at −20 °C. Within-batch precision was calculated from 20 determinations for each pool performed in the same assay, and between-day precision from determinations on 20 separate days.

Linearity. Linearity was determined by duplicate analyses of six dilutions with hormone-free serum of a serum containing a high concentration of progesterone. The hormone-free serum was prepared by adsorbing the endogenous hormones onto charcoal (*8*).

Recovery. Known amounts, determined spectrophotometrically, of progesterone (Sigma Chemical Co., St. Louis, MO 63178) were added to aliquots of hormone-free serum to produce sera containing 0, 2, 5, and 10 nmol of progesterone per liter. These pools were then assayed in duplicate, and the mean recoveries at each concentration calculated as a percentage of the expected value.

Accuracy. We analyzed in duplicate 100 patients' samples with each kit and compared the results with those obtained by our in-house method. Regression analysis was used to determine the relationship between each of the two kit methods and the comparison method, and a Student's *t*-test was used to assess the probability of a significant difference between the mean obtained by each kit method and that of the comparative method. We also analyzed in triplicate six quality-control sera (four commercial lyophilized preparations, two laboratory pools) and compared the means with the assigned values.

Sensitivity. Sensitivity, or detection limit—defined as the smallest concentration that can be distinguished from zero at the 95% confidence limit—was calculated from 2 SD of the mean of 25 replicate tests of the zero point of the standard curve.

Results

Standard curve. During the course of the evaluation we obtained 25 standard curves for each kit. For both kits, logit-log transformations of B/B_0 plotted against concentration were approximately linear. The lines generated with use of the DPC kit in general showed a poorer fit to the data points than those produced with the RSL kit (n = 25 standard curves. DPC: "mean r^2" = 0.9879, CV = 1.89%; RSL: "mean r^2" = 0.9954, CV = 0.44%).

Precision. The results obtained for within-batch and between-day precision are shown in Table 1. To be clinically useful at low concentrations of progesterone (<10 nmol/L), we considered that the between-day SD should not exceed about 0.5 nmol/L. At the low pool concentration of 3 nmol/L this would demand a CV of less than 17%. The RSL kit easily met this requirement, whereas the between-day CV for the DPC kit (22%) was unacceptable.

At progesterone concentrations of 20 nmol/L or higher, a between-day SD of 2 nmol/L is considered acceptable. This would require a CV of 10% or less at the medium pool concentration of 20 nmol/L. At this concentration of progesterone the precision of the RSL kit was satisfactory (between-days CV of 6.1%), but the performance of the DPC kit (CV = 17.5%) was unacceptable.

At extremely high concentrations of progesterone (around 100 nmol/L) a SD of 10 nmol/L, equivalent to a CV of 10%, would be acceptable. By this criterion, the DPC kit does not meet the desired level of precision.

To summarize, the DPC kit showed unacceptable precision at all three concentrations of progesterone tested, while both the RSL kit and the in-house ^3H method easily met the criteria for acceptable precision at the three concentrations of progesterone tested.

Table 1. Precision Data: Kits and Comparison Method

	Within-batch precision			Between-day precision		
	RSL	DPC	^3H[a]	RSL	DPC	^3H[a]
Low pool						
Mean, nmol/L	3.67	2.60	2.81	2.84	3.46	6.9
SD, nmol/L	0.37	0.34	0.22	0.35	0.77	0.70
CV, %	10.0	13.1	7.9	12.4	22.2	10.1
Medium pool						
Mean, nmol/L	20.54	25.40	41.4	21.55	25.20	15.6
SD, nmol/L	1.10	6.98	2.51	1.32	4.36	1.50
CV, %	5.4	27.4	6.1	6.1	17.3	9.6
High pool						
Mean, nmol/L	112.0	115.9	71.0	118.3	127.2	56.6
SD, nmol/L	4.61	10.01	5.23	6.88	17.27	3.85
CV, %	4.1	8.6	7.4	5.8	13.6	6.8

[a] Data for the in-house method were obtained from different serum pools before the evaluation of the kits.

As would be expected, within-batch precision was generally better than between-day precision for each method, except for the DPC kit, where the within-batch CV (27.4%) was significantly worse than the between-day CV (17.3%) at a progesterone concentration of 25 nmol/L.

Linearity. Results are shown in Figure 1. (The data shown for the in-house ^3H method were obtained from a different series of dilutions before evaluation of the kits.)

Errors for each method were calculated from the between-day precision data. These error limits were much wider for the DPC kit than for either the RSL kit or the in-house ^3H method, owing to the DPC kit's poor precision. For all methods the values obtained in the linearity studies fell within the respective allowable error limits, thus all were linear over the range tested (up to 60 nmol/L for the kits).

Analytical recovery. Both kits yielded recovery values (Table 2) that exceeded the values claimed by the manufacturers. The mean recovery for the RSL kit (116%) was similar to that for the in-house ^3H method (123%), and both were considered satisfactory for a steroid hormone assay.

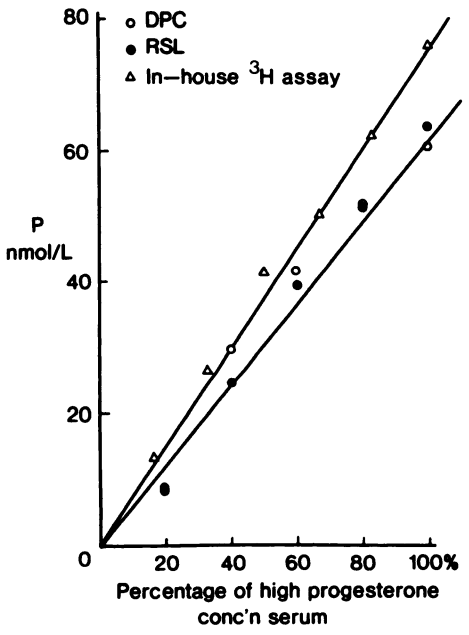

Fig. 1. Linearity study

Estimation by RSL, DPC, and ^3H in-house methods of various dilutions of a serum containing a high concentration of progesterone. The diluent was charcoal-stripped serum

Table 2. Analytical Recovery of Progesterone from Serum

	RSL kit		DPC kit		In-house ^{3}H method	
	Mean, nmol/L	Recovery, %	Mean, nmol/L	Recovery, %	Mean, nmol/L	Recovery, %
Serum pool	12.65	—	8.90	—	11.35	—
+ 2 nmol/L[a]	14.95	115	14.00	255	13.30	103
+ 5 nmol/L[a]	18.30	113	19.40	210	18.10	135
+ 10 nmol/L[a]	24.65	120	23.05	142	24.35	130
Mean recovery		116		202		123

[a] Added progesterone

The DPC kit, on the other hand, with a mean recovery of 202%, is clearly unacceptable.

Accuracy. Regression analysis of the test methods vs the comparison method demonstrated that both of the kits agreed well with the in-house ^{3}H method (Figure 2). There was no statistically significant ($p > .05$) difference between the means of the results obtained with either of the kits and those obtained with the comparison method.

Thus comparison of results from different laboratories and performance in quality-control programs should be largely unchanged with respect to accuracy if either of the kits were adopted for use instead of our current ^{3}H method.

Assigned values for the six quality control sera were obtained within the error limits allowable for each kit (based on between-day precision data, Table 3). No problems or potential problems associated with the use of lyophilized commercial quality control sera were noted.

Sensitivity. Sensitivity was 0.3 nmol/L for both kits. The manufacturer's claim for the DPC kit was 0.16 nmol/L; no value was stated for the RSL kit.

Duplicates. With the DPC kit we noted frequent poor agreement between cpm values for duplicate tubes. Almost half (45%) of the 465 duplicates differed by more than 5%, and 16% differed by more than 10% (22% and 1.5%, respectively, for the RSL kit). Many samples from each DPC assay would thus have to be repeated in actual practice, a costly exercise in terms of both time and money. Because the same operator carried out all the assays with both kits this poor agreement for duplicates is not due to differences between operators; moreover, the DPC kit requires fewer steps than the RSL kit. The most likely cause of the duplicates problem in the DPC kit is inconsistent antibody coating of the tubes. The frequent occurrence of bad duplicates could partly account for the poor precision obtained with this kit, and in particular for the anomalous result for within-batch precision at the medium concentration of progesterone.

Other considerations. The RSL kit requires four pipetting steps, a 37 °C water-bath, and centrifugation, whereas the DPC kit requires two pipetting steps, no water-bath, and no centrifugation. Instructions provided were considered to be adequate for both kits. The time taken to obtain results for

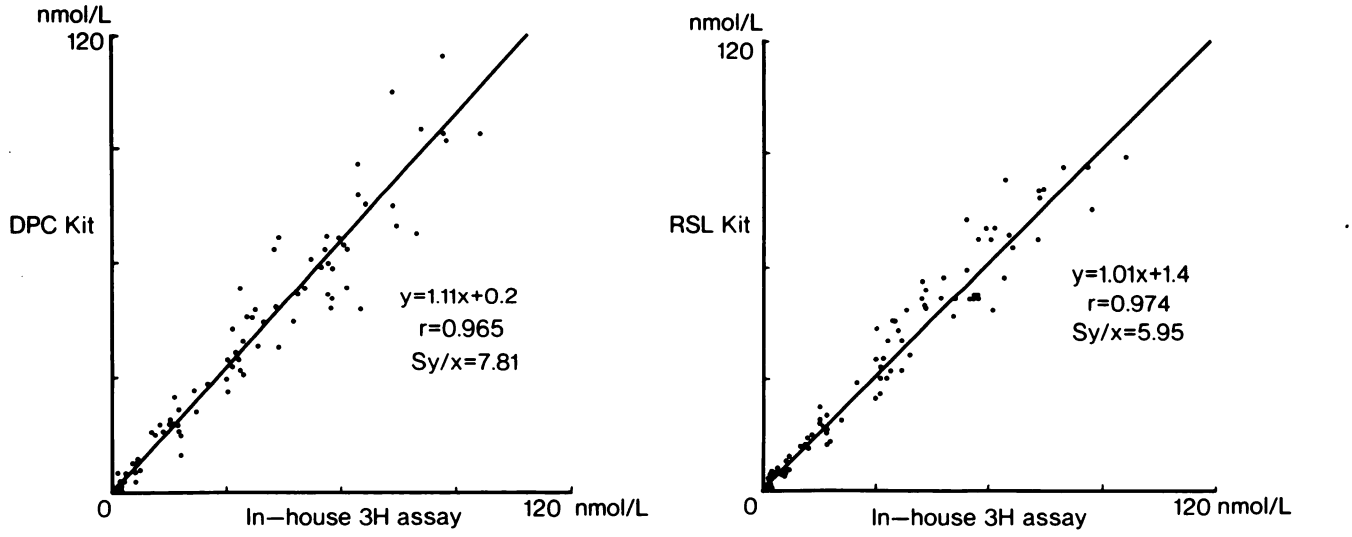

Fig. 2. Correlation of progesterone values obtained by using the DPC kit (*left*) and the RSL kit (*right*) with those obtained by use of the in-house ^{3}H method

Table 3. Progesterone Determinations in Quality-Control Materials

	Progesterone concn (mean ± SE), nmol/L			
Quality-control serum	Assigned value	RSL	DPC	In-house ^{3}H method
Steroid I Mallinckrodt	2.20 ± 0.15	2.6 ± 0.42	2.4 ± 0.92	3.8 ± 0.84
Ortho III Ortho Diagnostics	7.63 ± 1.91	9.1 ± 1.58	9.8 ± 5.23	7.8 ± 0.84
Steroid II Mallinckrodt	8.74 ± 0.39	9.0 ± 1.58	7.6 ± 5.23	12.2 ± 1.80
QC B In-house pool	15.6 ± 1.49	17.6 ± 1.58	15.8 ± 5.23	15.6 ± 1.49
Ortho IV Ortho Diagnostics	26.39 ± 6.36	26.7 ± 1.52	29.5 ± 5.23	28.3 ± 1.80
QC C In-house pool	56.6 ± 6.41	71.0 ± 8.26	68.7 ± 20.73	56.6 ± 6.41

20 unknown samples was approximately 3.5 h for the RSL kit and 4.25 h for the DPC kit, considerably shorter than the 24 h required for our ^3H method. Both kits thus meet the time requirement, bearing in mind that an assay for in vitro fertilization program purposes would usually contain far fewer than 20 samples.

Discussion

For general use in infertility investigations, where the turnaround time of biochemistry tests is not an important consideration, the major requirement of a progesterone assay is that it should be able to determine whether a cycle is in the luteal or follicular phase; therefore it should be precise at progesterone concentrations of approximately 20 nmol/L. The RSL kit showed good precision at 20 nmol/L and provided clinical information that corresponded to that provided by the ^3H-label assay. The RSL kit also demonstrated acceptable recovery, linearity, and sensitivity. For in vitro fertilization studies, which require good precision at progesterone concentrations of <10 nmol/L, only the RSL kit was acceptably precise.

The DPC kit, because of its poor precision at any of the concentrations evaluated, would incorrectly categorize samples as follicular or luteal approximately three times as often as the RSL kit. The results given by the DPC kit for accuracy and linearity studies appear acceptable, but the margins of error allowed were based on precision data and thus were very wide. Other drawbacks of the DPC kit were an unacceptably high analytical recovery and frequent poor agreement between duplicate tubes, which in turn probably partly accounts for the poor precision and recovery of the kit.

For these reasons, we conclude that the RSL kit would provide a rapid, precise, and accurate alternative to the standard extraction and β-counting method for measuring progesterone, whereas the DPC kit cannot be recommended.

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