Comparison of Three Methods for 17α -Hydroxyprogesterone

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We compared the performance of three 17α -hydroxyprogesterone kits: the double antibody method, the coated tube method (ACTIVE™), both from Diagnostic Systems Laboratories, Inc. (DSL) and the coated tube method (COAT-A-COUNT®) from Diagnostic Products Corporation (DPC). The assay performance of the two DSL kits was very similar in terms of sensitivity, intra- and inter-assay precision, linearity of dilution, recovery, and specificity. We also analyzed 190 samples for 17α -hydroxyprogesterone values using the above three kits. Twenty-three subjects were from prepubertal population (ages 1 month–13 years), thirty subjects were normal adult males (ages 20–53 years) and the remaining subjects were females in different phase of menstrual cycle ($n = 40$),

on oral contraceptives ($n = 20$), post-menopausal ($n = 17$), or pregnant women in their first, second, or third trimester ($n = 60$). In addition to these 60 pregnancy samples, we analyzed serial samples from 3 pregnancies. 17α -OHP levels paralleled the progesterone levels in all three kits. Although there was reasonable correlation between the DPC and the two DSL kits, the 17α -OHP values were found to be significantly higher with DPC kit during the 2nd and 3rd trimester of pregnancy indicating probable interference in the DPC assay by some structurally related steroids present during pregnancy. The DSL assays may be particularly well suited for measuring 17α -OHP levels during pregnancy. J. Clin. Lab. Anal. 11:179–185, 1997. © 1997 Wiley-Liss, Inc.

Key words: 17α -OH progesterone; progesterone; radioimmunoassay; normal values

INTRODUCTION

The steroid 17α -hydroxyprogesterone (17α -OHP, 17α -hydroxy-4-pregnene-3, 20-dione) is produced in the adrenal glands and gonads. In the adrenal glands, 17α -OHP is converted to cortisol through the sequential actions of 21-hydroxylase and 11-hydroxylase enzymes. Serum 17α -OHP levels are age-dependent (1–4), with peak levels observed during fetal life and the immediate postnatal period. During the first week of life, serum 17α -OHP levels fall ~50-fold as compared to cord blood values. A small transient increase occurs in male infants 30–60 days postnatally. Levels for both sexes then remain at constant and comparable low levels during childhood and progressively increase during puberty, reaching adults levels of ~1 ng/mL. As with cortisol (5), serum 17α -OHP levels normally have an ACTH-dependent diurnal variation, with peak levels in the morning and a nadir at night. In addition, ovarian production of 17α -OHP increases during the luteal phase of the menstrual cycle, leading to serum levels which are several fold higher than during the follicular phase (6, 7).

Measurement of circulating 17α -OHP levels is considered a standard tool for clinical assessment of 21-hydroxylase de-

ficiency, the most common cause of congenital adrenal hyperplasia (CAH) (3, 4, 8). Because 17α -OHP levels are markedly elevated in neonatals and adolescent girls with CAH, a single basal measurement is generally sufficient to make the diagnosis (9, 10). In addition, elevated 17α -OHP levels in response to parenteral administration of ACTH have been used to diagnose “partial” 21-hydroxylase deficiency, a potential cause of female hirsutism and infertility (8).

Several immunoassay methods for determining 17α -OHP levels have been described in the literature (11) that use antibodies raised against 17α -OHP-protein conjugates along with tracer molecules containing enzyme labels (12, 13), fluorescent labels (14), or ^{125}I labels. These methods permit measurement of the analyte in small volumes of blood spots (15, 16), serum or saliva (17, 18). However, in some cases, the presence of both known and unknown cross-reacting steroids

Nonstandard abbreviations: 17α -OHP, 17α -hydroxyprogesterone; CAH, Congenital adrenal hyperplasia.

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may necessitate laborious sample extraction and chromatographic steps prior to assay. In recent years, direct assays with antibodies having high specificity to this hormone have been made available in kit formats by several manufacturers (19). In this paper, we (a) report performance characteristics of two DSL (Diagnostic Systems Laboratories, Webster, Texas 77598) kits (double antibody, DAb and coated-tube, ACTIVE™), (b) compare the DPC (Diagnostic Products Corporation, Los Angeles, CA 90408) COAT-A-COUNT® kit with the two DSL kits by measuring 17 α -OHP values in 190 normal subjects in the following category: (1) prepubertal children, (2) normal males, (3) women with validated normal menstrual cycles, (4) women on oral contraceptives, (5) postmenopausal women, and (6) pregnant women.

MATERIALS AND METHODS

Sample Population

Prepubertal children are categorized by age and had no known endocrine disorders, nor were they on any endocrine medication. Male subjects were apparently healthy volunteers and were not on any medication. Menopausal women in this study included women with normal regular menstrual cycles and without any symptoms of hyperandrogenism. They were arbitrarily divided into luteal and follicular phase on the basis of cycle length and endocrine status. A group of women on oral contraceptives was also investigated. Postmenopausal women were those who did not have a regular menstrual cycle for at least 9–12 months and who were in their late 40's to early 50's. Cross-sectional samples were obtained from pregnant women at the 1st, 2nd, and 3rd trimester. Serial pregnancy samples were obtained from individual pregnant women at regular intervals throughout the course of gestation. All of the pregnant women in this study had normal singleton pregnancies and all endocrine values were essentially in the normal range in these women.

Immunoassays

Samples were analyzed in duplicate and the data reduction was performed using log-logit transformation. The kits were used in between the beginning to middle of their shelf-life.

A. DSL Assays. Both kits (DAb: DSL-6800 and ACTIVE™: DSL-5000) use rabbit anti-17 α -OHP serum. The manufacturer's instructions were followed in running both the kits. Briefly, for DAb assay, 50 μ L of 17 α -OHP standards, controls, or unknowns were incubated with 500 μ L ¹²⁵I-labeled 17 α -OHP and 100 μ L 17 α -OHP antiserum at 37°C for 1 hour. Following the addition of 1 mL precipitating reagent and subsequent centrifugation, tubes were decanted and counted in a gamma counter. For ACTIVE™ assay, anti-17 α -OHP IgG coated tubes were incubated with 50 μ L standards, controls or unknowns in the presence of 500 μ L ¹²⁵I-labeled 17 α -OHP for 1 hour at 37°C, decanted and counted in a gamma counter. The Dynamic range of both assays are 0–20 ng/mL.

B. DPC COAT-A-COUNT® Assay. The manufacturer's instructions were followed in running this kit. 25 μ L standards, controls, or unknowns were incubated with 1.0 mL ¹²⁵I-labeled 17 α -OHP in plastic tubes coated with 17 α -OHP antibody. After 3 hours at RT, the tubes were decanted and counted in a gamma counter. Dynamic range of the DPC assay is the same as that of the DSL assays.

RESULTS

A. Performance Characteristics

- 1. Sensitivity.** The theoretical limits of sensitivities for the DPC and DSL assays as determined by interpolating the mean minus 2SD of 12 replicates of the zero standard are 0.02, 0.014, and 0.08 ng/mL for DSL, DAb, DSL ACTIVE™, and DPC assays respectively.
- 2. Precision.** The reliability of DPC, DSL DAb, and DSL ACTIVE™ 17 α -OHP assay procedures was evaluated by examining their reproducibility on samples chosen to represent a range of 17 α -OHP levels. The precision of the DPC and two DSL assays is shown in Table 1. Intra-assay precision of the DPC and two DSL assays is shown in Table 1. Intra-assay precision was determined by assaying 12 replicates of patient samples in single assays. Inter-assay precision was assessed by performing repeat analysis of the samples in 12 different runs during one week period. As indicated in Table

TABLE 1. Precision of DPC, DSL DAb, and DSL ACTIVE™ Assays

Sample	DSL DAb		DSL ACTIVE™		DPC	
	Mean $\pm$ SD (ng/mL)	CV %	Mean $\pm$ SD (ng/mL)	CV %	Mean $\pm$ SD (ng/mL)	CV %
Intra-Assay						
I	1.06 $\pm$ 0.06	5.9	1.18 $\pm$ 0.11	9.3	1.24 $\pm$ 0.05	4.5
II	3.85 $\pm$ 0.16	4.1	4.67 $\pm$ 0.38	8.1	6.29 $\pm$ 0.25	4.0
III	9.04 $\pm$ 0.34	3.7	9.08 $\pm$ 0.86	9.5	20.0 $\pm$ 2.1	10.6
Inter-Assay						
I	1.00 $\pm$ 0.08	7.9	1.13 $\pm$ 0.11	9.7	0.84 $\pm$ 0.09	11.1
II	4.04 $\pm$ 0.24	6.0	4.77 $\pm$ 0.30	6.3	4.15 $\pm$ 0.27	6.4
III	9.15 $\pm$ 0.29	3.2	9.43 $\pm$ 1.02	10.8	7.67 $\pm$ 0.35	4.5

1, the DSL DAb assay exhibited the highest precision, whereas the coated tube assays (DSL ACTIVE™ and DPC Coat-A-Count®) showed slightly higher CVs.

3. **Linearity of dilution.** Three serum samples with low, medium, and high endogenous levels of 17 α -OHP were diluted serially with 0 ng/mL 17 α -OHP standard and assayed in the two DSL kits. The expected values were plotted against the observed values as shown in Figure 1. The slopes of regression equation and "r" values were found to be close to 1.0, indicating that both assay procedures maintain good linearity of dilution.
4. **Spiking Recovery.** Three serum samples containing different levels of endogenous 17 α -OHP were spiked with known quantities of 17 α -OHP and assayed in the DPC as well as both DSL 17 α -OHP kits. The results presented in Table 2 demonstrate the accuracy of the three kits.
5. **Specificity.** The cross-reactivity of the 17 α -OHP antiserum was measured against various steroids in the DSL assays. The percent cross-reactivity is expressed as the ratio of the 17 α -OHP concentration to the concentration of the reacting steroid at 50% binding of the zero calibrator. Table 3 shows cross-reactivities of antisera used in making the two DSL kits and the DPC kit to some of the naturally occurring steroids that may be present in patient samples. Cross-reactivities to various other steroids were found to be negligible.

Fig. 1. Linearity of dilution of 17 α -OHP in serum samples using the DSL kits. The observed values were between 86–115% of the expected values.

B. Sample Comparison

All results are expressed as ng/mL. To convert to nmol/L:

$$\text{ng/mL} \times 3.03 = \text{nmol/L}$$

1. **Prepubertal Children.** When serum samples from prepubertal children ranging from 1 month to 13 years of age were compared between the two DSL kits and, among DPC and DSL kits, excellent correlation was observed, as shown in Figure 2. We also separated the prepubertal population of 31 samples total, into two groups: 1–11 months ($n = 14$) and 1–13 years ($n = 16$) and compared 17 α -OHP values between the two DSL kits. Figure 3 demonstrates the normal postpartum decline in levels of 17 α -OHP with increasing age and a parallel performance of both DSL kits.
2. **Normal Males.** When serum samples for 19 males (20–53 years of age) were compared in the three kits, mean $\pm$ SE for each kit was as follows: DSL ACTIVE™: 1.56 ± 0.16 ng/mL; DSL DAb: 1.57 ± 0.14 ng/mL; DPC COAT-A-COUNT®: 158 ± 0.13 ng/mL.
3. **Females.** Serum samples from women with validated normal menstrual cycle (follicular and luteal phase) and women on oral contraceptives, postmenopausal and pregnant women were evaluated for 17 α -OHP levels using the three kits. Results are tabulated in Table 4.

These data reflect a general positive correlation between the progesterone and 17 α -OHP levels. Thus, in states or phases where lower levels of progesterone would be expected, e.g. follicular phase, women on oral contraceptives, or postmenopausal women, the 17 α -OHP levels are also low; the mean 17 α -OHP values are similar for all three kits but are the lowest for DPC kit in these states. On the other hand, during luteal phase and pregnancy, where progesterone levels are elevated, the corresponding 17 α -OHP values are also high; the mean 17 α -OHP values are similar for both DSL kits but are found to be significantly higher for the DPC kit in pregnant women. Figure 4 compares the 3rd trimester 17 α -OHP values for all the three kits.

It is apparent that in each trimester as the progesterone values increase, there is a greater discrepancy in the correlation of DSL tests with DPC results. In order to highlight this discrepancy, 17 α -OHP levels were measured with all three assay methods in serial sets of samples through three individual pregnancies. As shown in Figure 5, the 17 α -OHP levels in the DPC assay increase above DSL results in the second half of pregnancy when the levels of progesterone are increasing. Progesterone levels in all the three serial pregnancies were measured using DPC COAT-A-COUNT® assay.

4. **Entire Sample Population.** Figure 6 represents the method comparison of the entire sample population measured in all three assays ($n = 190$). As evident from the equations, the two DSL kits show excellent correlation

TABLE 2. Recoveries of Spiked Serum Samples With DLS and DPC assays

Sample	Endogenous (ng/mL)	Added (ng/mL)	Expected (ng/mL)	Observed (ng/mL)	Recovery (%)
A. DSL DAb Assay					
I	0.54	0.31	0.85	0.81	95
		3.65	4.19	3.76	90
		9.33	9.87	9.12	92
II	1.87	0.31	2.18	2.38	109
		3.65	5.52	5.30	96
		9.33	11.20	11.73	105
III	4.54	0.31	4.85	4.69	97
		3.65	8.19	7.10	87
		9.33	13.87	14.57	105
B. DSL ACTIVE™ Assay					
I	2.29	0.22	2.51	2.39	95
		0.63	2.92	3.14	108
		3.66	5.95	7.00	118
II	4.25	0.22	4.47	4.53	101
		0.63	4.88	5.60	115
		3.66	7.91	6.83	86
III	6.85	0.22	7.07	7.26	104
		0.63	7.48	7.56	101
		3.66	10.51	10.73	102
C. DPC Coat-A-Count® Assay					
I	0.62	0.25	0.87	0.72	83
		0.75	1.37	1.25	91
		3.80	4.42	4.49	102
II	3.03	0.25	3.28	2.97	91
		0.75	3.78	3.49	92
		3.80	6.83	6.24	91
III	3.30	0.25	3.55	3.98	112
		0.75	4.05	4.47	110
		3.80	7.10	7.35	104

(Figure 6A). On the other hand, there is a clear shift in the regression line towards the DPC side (Y-axis) when the DPC kit is compared with the two DSL kits; however, the overall correlation ($r = 0.871$) between the two DSL vs. DPC kits is good (Figures 6B and 6C).

DISCUSSION

The two DSL kits as well as the DPC kit measure 17α -OHP levels in patient samples without requiring extraction. The dynamic range of all three assays is identical. However, the DSL kits exhibit higher sensitivity and require less turn-around time compared to the DPC kit. The DSL and DPC coated-tube assays are convenient and simple to perform, but

the DSL ACTIVE™ assays offers the advantage of shorter incubation time compared to the DPC COAT-A-COUNT® assay (1 hour vs. 3 hours).

Both DSL assays showed acceptable intra- and inter-assay coefficients of variation, the DAb kit showing slightly better precision. Linearity of dilution studies showed recovery of 86–115% for DAb assay and 85–115% for ACTIVE™ assay. Recovery of 17α -OHP added to serum samples ranged from 87–105% for DAb assay and 86–115% for ACTIVE™ assay. Cross-reactivity studies suggests that antisera used in all three kits are highly specific for 17α -OHP with the DSL DAb kit showing the highest specificity.

As expected, the prepubertal population showed a general

TABLE 3. Cross-reactivities of DSL and DPC Antisera

Sample	% Cross-reactivity		
	DSL DAb assay	DSL ACTIVE™ Assay	DPC Assay ^a
Progesterone	0.72	2.3	3.5
11-deoxycortisol	0.57	2.0	1.5
17α -OH-Pregnenolone	0.69	ND	0.14
Pregnenolone	ND	ND	0.14
5α -pregnan-3,20-dione	ND	ND	0.60

^aManufacturer's reported cross-reactivities.

ND, non-detectable (<0.01%)

Fig. 2. Regression analysis of prepubertal samples using two DSL kits and DPC kit.

trend of declining levels of 17 α -OHP with age. Excellent correlation was observed between the two DSL kits, and between the DPC and DSL kits (Figure 2). The 17 α -OHP levels measured in normal adult male population with these three kits were almost identical.

Fig. 3. Measurement of 17 α -OHP levels in prepubertal children with increasing age, with DSL DAb and DSL ACTIVE™ 17 α -OHP kits. There exists an inverse relationship between prepubertal age and 17 α -OHP levels.

In females, during the follicular and luteal phases of the menstrual cycle, the 17 α -OHP levels measured with the three kits were very similar, but were about 4-fold higher in the luteal phase. This can be attributed to the increased levels of progesterone during the luteal phase. Serum samples from postmenopausal women and women on oral contraceptives exhibited overall low levels of 17 α -OHP, with the DSL ACTIVE™ kit showing slightly higher values than the DSL DAb or DPC COAT-A-COUNT® assays.

During pregnancy, levels of 17 α -OHP were evaluated from the first to the second to the third trimester in 60 cross-sectional samples (Table 4). We also studied three serial pregnancy samples in order to gain information about the pattern of 17 α -OHP levels throughout the entire pregnancy. In the three longi-

TABLE 4. Comparison of 17 α -OHP Levels in Females Using DSL DAb, DSL ACTIVE™, and DPC COAT-A-COUNT® Kits

Sample Population	N	Mean $\pm$ SE (ng/mL)		
		DSL DAb	DSL ACTIVE™	DPC COAT-A-COUNT®
Follicular Phase	18	0.57 $\pm$ 0.03	0.65 $\pm$ 0.04	0.45 $\pm$ 0.04
Luteal Phase	22	2.52 $\pm$ 0.14	2.66 $\pm$ 0.19	2.96 $\pm$ 0.23
Oral Contraceptives	20	0.34 $\pm$ 0.03	0.47 $\pm$ 0.05	0.28 $\pm$ 0.02
Post menopausal	17	0.48 $\pm$ 0.06	0.61 $\pm$ 0.07	0.42 $\pm$ 0.05
1st Trimester	20	3.02 $\pm$ 0.25	3.07 $\pm$ 0.24	4.30 $\pm$ 0.41
2nd Trimester	20	4.10 $\pm$ 0.43	4.68 $\pm$ 0.47	7.38 $\pm$ 0.81
3rd Trimester	20	6.36 $\pm$ 0.50	7.27 $\pm$ 0.57	11.60 $\pm$ 0.94

Fig. 4. Regression analysis of third-trimester pregnancy samples using DSL DAb, DSL ACTIVE™ and DPC COAT-A-COUNT® tubes.

tudinal serial samples, a decline in 17α -OHP levels was observed during the early part of the first trimester, levels then remained steady during the second trimester and elevated moderately, late in the third trimester with the two DSL kits; the 17α -OHP values obtained with the DSL kits are in agreement with the values reported in the literature (20). In contrast, the 17α -OHP levels measured with the DPC kit were always significantly higher in cross-sectional as well as longitudinal samples (Table 4, Figures 4 & 5). This discrepancy may arise due to the possible existence of greatly elevated concentrations of several steroids and their numerous uncharacterized metabolites (e.g., pregnenolone, 17α -hydroxypregnenolone, pregnenolone sulfate, 16α -hydroxyepiandrosterone, and several other 3β -hydroxy-5-ene steroid metabolites) during pregnancy, some of which may interfere/cross-react in the DPC assay causing the 17α -OHP values to increase in the DPC assay.

Fig. 5. Comparison of 17α -OHP in serial samples from three pregnancies using DSL DAb, DSL ACTIVE™ and DPC COAT-A-COUNT® radioimmunoassay. Progesterone values are presented for reference comparison.

When the entire sample population ($n = 190$) was used to compared all three methods, the two DSL kits correlated very well (Figure 6A). Comparison of samples between the DPC kit and the two DSL kits (Figures 6C & 6C) showed reasonable correlation but overall, samples read higher with the DPC kit. Some outliers were observed to the right of the regression line in Figures 6B and 6C. These points represent neonatal samples between the ages of 1–3 months which read significantly higher in the two DSL kits. In retrospect, when Makela and Ellis (21) evaluated and compared the DPC kit with their in-house method in neonatal serum samples of less than six months of age, they observed elevated 17α -OHP levels with the DPC kit as compared to their in-house method. The higher neonatal values observed with the DSL and DPC kits may be attributed to some uncharacterized steroids and/or their metabolites (21) present in elevated concentrations only in the neonatal serum matrix; these compounds may interfere/cross-react with the DSL and DPC assays to varying degrees in neonatal samples.

In conclusion, although the overall performance of the DSL and DPC kits is comparable, the DSL kits offer advantages in terms of shorter incubation time, higher sensitivity, and probably less interference from other structurally related ste-

Fig. 6. Regression analysis of the entire sample population using DSL DAB, DSL ACTIVE™, and DPC COAT-A-COUNT® tubes.

roids during pregnancy. Thus, the DSL kits may be a better choice if one were to study the endocrinology of pregnancy. Further studies are required to clarify the comparison in the neonatal population, and evaluate the utility of these kits in measuring neonatal 17 α -OHP values.

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