Nonspecificity of a Direct 17α-Hydroxyprogesterone Radioimmunoassay Kit When Used with Samples from Neonates

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We analyzed 240 samples for 17α-hydroxyprogesterone (17-OHP) with the direct-assay kit ("Coat-A-Count" method for serum samples) from Diagnostic Products Corp. (DPC). The specimens were from 50 patients known or suspected of congenital adrenal hyperplasia (CAH); 74 mostly hospitalized neonates and infants, ages three days to three months; and 116 other patients, ages six months to 23 years. Samples from the CAH group were also analyzed with our in-house assay. For 39 of the neonatal samples, the analysis with the DPC assay was repeated with re-solubilized material that had been extracted from the serum with organic solvents. Values for "17-OHP" measured with the DPC direct assay were high, not only in CAH patients, but also in many of the unaffected neonates and infants. The extraction properties of the cross-reacting immunoreactive material into various organic solvent systems were different from those of 17-OHP, and were more like those of steroid sulfates. Because of this significant cross-reactivity, we recommend that the DPC kit not be used for sera from children younger than six months of age, unless the method is modified to include an extraction step.

Additional Keyphrases: analytical error • congenital adrenal hyperplasia • extraction methods • pediatric chemistry • steroids

The term "congenital adrenal hyperplasia" (CAH) covers a group of inborn errors of steroid biosynthesis (1–4). The commonest subgroup is 21-hydroxylase deficiency, in which 21-hydroxylase enzyme activity is partly or almost completely deficient. Consequently, the capacity to synthesize cortisol is decreased, and corticotropin feedback and androgen production are increased. The steroid substrate for the 21-hydroxylase enzyme, 17α-hydroxyprogesterone (17-OHP), is increased in serum or saliva (5, 6) from affected subjects. Its measurement is useful for diagnosing the condition and for monitoring compliance or efficacy of steroid therapy. Like cortisol, it shows a diurnal variation in its concentrations. Siblings of known affected subjects can be tested a few days after birth, before salt loss becomes a problem in patients from families that are at risk for this. These measurements can confirm conclusions derived from antenatal testing by histocompatibility antigens (HLA) or gene-probe typing of chorionic villus (7) or by steroid measurements in amniotic fluid (1, 8).

Assays of 17-OHP in serum should be suitable for patients of all ages, especially those with classical CAH, and a routine assay must be especially effective in analyzing sera from neonates and infants younger than two months if salt-wasting crises or misdiagnosis are to be avoided.

Methods for determining the concentration of 17-OHP in serum have been reviewed (9). In early competitive protein binding assays for 17-OHP, serum was the source of corticosteroid-binding globulin. Because of the nonspecificity of the corticosteroid-binding globulin, organic-solvent extraction of the sample to be tested and subsequent purification of the extract by chromatography often preceded the competitive protein binding assay. Radioimmunoassays involving more specific antibodies still required an extraction step, to improve assay specificity and to concentrate the steroid from the sample. More recently, direct assays were described that use antibodies with sufficient specificity and affinity, combined with enzyme labels (10, 11), fluorescent labels (12), or high-activity 125I-labeled tracers to permit the direct assay of small volumes of serum, saliva (13, 14), or blood spots (15, 16). Direct assays have also been developed by commercial companies and are available in kit form. We report our experience with kits from DPC (Diagnostic Products Corporation, Los Angeles, CA 90403) and draw attention to the deficiencies of this kit for measuring 17-OHP in serum during the first six postnatal months.

Patients and Methods

Patients

Group A: neonates and infants. This group consisted of 74 infants, ages three days to three months, who were admitted to our neonatal intensive-care wards or who were attending the hospital as outpatients. Physicians had requested routine blood work such as electrolytes at various times during the day, and surplus plasma or serum from these specimens was stored in aliquots at −20°C until assay for 17-OHP. All case histories were reviewed and patients were eliminated from the study if they were receiving therapy with steroids or had adrenal disorders. The patients had various diagnoses typical of a tertiary-care neonatal facility. Many were premature, low-birth-weight infants with respiratory distress syndrome. Many had infections. Some had congenital abnormalities. Their condition ranged from stable to severely ill. Seven of the outpatients were babies being treated with thyroxin for neonatal hypothyroidism detected by a screening program.

Group B: older infants, children, and young adults. This group consisted of 116 individuals, ages six months to 23 years. Most were outpatients, but some were inpatients on general pediatric wards. Surplus serum or heparinized plasma (obtained at various times of the day), available after routine thyroid-function tests were completed, was stored at −20°C until tested for 17-OHP. Again, patients were eliminated from the study if they were receiving therapy with steroids or had any adrenal disorders. These patients had a wide range of diagnoses. The most common were follow-up of diabetes mellitus (16), hypothyroidism (12), gastrointestinal conditions (10), chromosomal abnormalities (8), cancers (7), and neurological conditions (7).
Group C: neonates and infants. These 38 children were similar to those in group A, but their case records were not examined. Their sera or plasmas were pooled for many of the studies.

Group D: congenital adrenal hyperplasia. The group consisted of 50 patients (mainly outpatients) older than six months, whose doctor had requested 17-OHP assay for the diagnosis or monitoring of CAH. Samples were taken at various times of the day. Most patients in this group had CAH and were receiving therapy with steroids, but in many cases compliance or dosage was unsatisfactory. Serum or plasma was assayed for 17-OHP by the routine in-house method, then stored at \(-20 \, ^\circ\text{C}\) until assay with the kit from Diagnostic Products.

Procedures

In-house method with tritiated 17-OHP. Extract 200 \(\mu\)L of serum or plasma with 2 \(\mu\)L of a 30 mL/L solution of 1-propanol in heptane. Shake vigorously for 2 min in a screw-capped glass vial, then centrifuge for 2 min at \(1400 \times g\) to separate the phases. Transfer 500-\(\mu\)L and 50-\(\mu\)L aliquots, each in duplicate, to four 12 \(\times\) 75-mm glass tubes. Evaporate the solvent under flowing nitrogen. Add to the extract 100 \(\mu\)L of phosphate buffer (0.1 mol/L, pH 7.5) containing 5 g of bovine serum albumin per liter (BSA-buffer). Vortex-mix thoroughly.

Prepare a set of seven standard tubes, in duplicate, containing, per tube, 0-0.5 ng of 17-OHP (Sigma Chemical Co., St. Louis, MO 63178; cat. no. H5752) in BSA-buffer.

Add 100 \(\mu\)L (50 000 dpm) of \([^{3}\text{H}]17\)-OHP (New England Nuclear, Boston, MA 02118; prod. no. NET-332) in BSA-buffer to all standard and sample tubes and to two "total-count" tubes. Add 100 \(\mu\)L of 17-hydroxyprogesterone antibody (Bio-Mega Diagnostic Inc., Montreal, Quebec H3M 3A2 Canada), raised against 17\(\alpha\)-hydroxyprogesterone-7-thiopropionate-bovine serum albumin, and diluted in BSA-buffer containing 20 mL of normal rabbit serum per liter. The appropriate antibody dilution should give 50% binding of tracer at \(B_0\). Vortex-mix and incubate for 1 h at room temperature, then 1 h at 0-4 \(^\circ\text{C}\). Add 1 mL of a cold (5-4 \(^\circ\text{C}\)) well-mixed suspension of affinity-purified goat anti-rabbit gamma globulin (Joldon Diagnostics, Agincourt, ON M1V 1E7 Canada) in BSA-buffer containing 40 g of polyethylene glycol 8000 (J. T. Baker Chemical Co., Phillipsburg, NJ 08865) per liter. The goat gamma-globulin dilution should be appropriate to completely precipitate the gamma-globulins in 2 \(\mu\)L of normal rabbit serum per tube (as determined by previous titration of the specific lot number). Vortex-mix, let sit at 0-4 \(^\circ\text{C}\) for 30 min, then centrifuge at 1400 \(\times\) \(g\) for 20 min.

Decant the supernate into 20 mL glass liquid-scintillation vials. Add 12 mL of "PCS" liquid-scintillation cocktail (Amersham Canada Ltd., Oakville, ON L6H 2R3 Canada). Mix well, and count the radioactivity for 2 min in a liquid-scintillation counter (we used an LKB "Rack Beta"). Enter the standard counts into a suitable data-reduction program and calculate values for the unknowns (we used a Scatchard plot to calculate the data). If the counts for the 500-\(\mu\)L extract lie on the standard curve, use this value to calculate the result. If the counts exceed that for the 0.5 ng per tube standard, use the counts corresponding to the 50-\(\mu\)L extract. Repeat the assay with smaller volumes of extract for sera with unusually high concentrations of 17-OHP. In each case, allow for the dilution factor introduced by taking a fraction of the total extract of the serum.

The percentage cross-reactivity of the antibody with various steroids is: progesterone 0.06, 11\(\alpha\)-deoxycorticosterone 0.05, 17\(\alpha\)-hydroxyprogrenolone 0.02; and pregnenolone, testosterone, dihydrotestosterone, androstenedione, androstan-3\(\alpha\),17\(\beta\)-diol, estradiol, estrone, and dehydroepiandrosterone all <0.01 (manufacturer’s data).

Diagnostic Products Corporation’s Coat-A-Count method (DPC method). We used the Coat-A-Count kit for 17-OHP (cat. no. TK OP1; Diagnostic Products Corp.) for serum samples as directed by the manufacturer. This kit is an antibody-coated-tube method with high specific activity 125\(\text{I}\) tracer. Serum (25 \(\mu\)L per tube) is assayed directly, i.e., without extraction or chromatography. The antibody is very specific. Of 62 steroids and drugs tested by the manufacturer, only the following appeared to show slight cross-reactivity (%): 11-deoxycortisol (1.0), 17\(\alpha\)-hydroxyprogrenolone (0.4), progesterone (0.07), and pregnenolone (0.06). The cross-reactivities of the remaining compounds were all <0.03%.

Coat-A-Count method with extraction (DPC method with extraction). Extract 200 \(\mu\)L of serum or plasma with 2 mL of propanol/heptane as with the in-house method. Transfer 1.0 mL of each extract to a 12 \(\times\) 75-mm glass tube and evaporate the solvent under flowing nitrogen. Reconstitute the extract with 100 \(\mu\)L of zero calibrator from the kit. Vortex-mix thoroughly three times, with a few minutes between each mixing. Use 25 \(\mu\)L of reconstituted extract in place of serum in the DPC method.

Efficiency of extraction with propanol/heptane. Method 1: Assay human serum-based DPC quality-control serum by the DPC method, with or without extraction.

Method 2: Prepare a pool of normal serum. Evaporate the solvent from 2 \(\mu\)Ci of \([^{3}\text{H}]17\)-hydroxyprogesterone in ethanol contained in a glass vial. Add 10 mL of pooled serum, with mixing. Cap the vial and leave at 0-4 \(^\circ\text{C}\) overnight, then extract 200-\(\mu\)L aliquots with 2 mL of solvent and evaporate 1 mL of the organic extracts in glass scintillation vials. Add to the residue 100 \(\mu\)L of the original (unlabeled) pooled serum and 10 mL of scintillant. Compare the counts in this vial with those of a vial with 100 \(\mu\)L of tritiated pool and 10 mL of scintillant.

Diethyl ether extraction of serum. Extract 200 \(\mu\)L of serum by shaking vigorously for 5 min with 4 mL of diethyl ether, in a screw-capped glass vial. Freeze the aqueous phase in a solid CO\(_2\)-acetone mixture, decant the supernate, and evaporate under flowing nitrogen. Reconstitute in 200 \(\mu\)L of DPC zero calibrator and take 25 \(\mu\)L for assay in the DPC method.

Extraction of serum with diethyl ether/ethanol in the presence of ammonium sulfate. Extract 200 \(\mu\)L of pooled plasma from neonates (group C) by shaking vigorously in a capped glass vial with 300 \(\mu\)L of 5 mol/L aqueous ammonium sulfate solution and 2 mL of diethyl ether/ethanol (3:1 by vol). Freeze the aqueous phase in a solid CO\(_2\)-acetone bath, pour off the organic phase, evaporate it under flowing nitrogen, and reconstitute the residue in 200 \(\mu\)L of DPC zero calibrator. Assay 25 \(\mu\)L of reconstituted material in the DPC assay.

Ultrafiltration of serum. Ultrafilter 400 \(\mu\)L of pooled serum from neonates and infants (group C) through an "Ultra-free" calcium filter (product no. DR 0063100; Worthington Diagnostic Division, Freehold NJ 07728). Assay the original pool, the ultrafiltrate, and the residual material after ultrafiltration, using the DPC method.

Treatment of serum with \(\beta\)-glucuronidase (EC 3.2.1.31) and sulfatase (EC 3.1.6.1). Acidify 1 mL of pooled serum.
β-glucuronidase (Type H-1; Sigma Chemical Co., St. Louis, MO) representing 900 Fishman (Sigma) units of glucuronidase and 44 U of sulfatase from Helix pomatia. Incubate at 37 °C for 24 h. Assay the serum directly with the DPC kit along with reconstituted extracts after extraction with 90 mL propanol in heptane, diethyl ether, dichloromethane, or diethyl ether/ethanol/ammonium sulfate.

**DPC neonatal 17-OHP kit for blood spots.** DPC markets a kit (cat. no. TKN1P1) for analysing 17-OHP in spots of blood dried (on filter paper) from neonates. The antibody used with this kit is the same as that used in the serum assay kit.

We prepared blood spots by mixing equal volumes of plasma from neonates and infants from group C with the corresponding residual packed cells and pipetted 75 μL slowly onto Schleicher & Schuell no. 903 filter paper (the paper used by the Ontario Ministry of Health and by many other regional screening programs). This avoided the need to take blood from the infants for study purposes, but approximates blood drawn directly onto filter paper. We allowed the filter paper to dry at room temperature for 4 h, then punched 30-mm discs from the paper and from the blood spot standards provided with the kit. We completed the assay according to the manufacturer's instructions. The results were calculated as nanomoles per liter of serum, assuming a 55% hematocrit (as recommended by the manufacturer).

**Results**

Some Analytical Variables

**Effectiveness of the extraction procedure.** This was assessed as described in the Methods section. Analytical recoveries were 89.2% (n = 5) by method 1, 87.6% (n = 10) by method 2.

**Correlation between the DPC and in-house methods.** Figure 1 shows the correlation between the DPC and in-house methods for patients in group D. This correlation includes normal individuals and patients with CAH who were being treated with steroids. Correlation was fair at low 17-OHP concentrations but poor at higher ones. From 26 of these samples sufficient serum was available to permit assay by the DPC method with extraction. Results obtained by the DPC method with extraction (y) correlated better with those by the in-house method (x), the equation for the correlation being y = 0.50x + 1.97 (r = 0.933, S_yp = 3.60), but about half the immunoreactive material was not accounted for in the extract.

**Imprecision of the DPC method.** The imprecision of the DPC method was assessed by use of DPC quality-control sera. Results are shown in Table 1.

**Reference Values for Children and Young Adults by the DPC Method**

Sera from the patients in groups A and B were assayed by the DPC method (Figure 2). Values in children younger than six months are higher than those for older children and young adults. A cumulative frequency plot was prepared for the 88 male and 63 female infants and children over six months of age (group B). The data fitted a log-gaussian distribution with the 97.6th centile at 6.3 nmol/L. In comparison, values are 5.4 nmol/L for men and 9.9 nmol/L for women, as quoted by the manufacturer.

Figure 3 shows the lack of a simple relationship between 17-OHP as measured with the DPC kit and gestational or chronological age in the neonates and infants younger than three months.

**Nature of the Substances in Neonates’ and Infants’ Plasma Measured as 17-OHP with the DPC Kit**

**Extractability into propanol–heptane.** Thirty-nine plasma samples from group A were tested undiluted and results were compared with those for the corresponding reconstituted extracts. The mean value by the DPC method with extraction was 3.3 nmol/L. For 12 of these samples, the DPC method without extraction gave values greater than that for
the highest calibrator, 60.5 nmol/L. After omitting these results, the mean 17-OHP value was 18.6 nmol/L without extraction and 1.8 nmol/L with extraction (Table 2).

Extractability into diethyl ether. Sera from two patients in group A (neonates) and two from group D (CAH) were assayed both directly by the DPC method and on reconstituted residues obtained after extraction with either propane/heptane or diethyl ether. The immunoreactive material in babies’ plasma was much less extractable by organic solvents than that in CAH plasma (Table 2).

Extractability into diethyl ether/ethanol in the presence of ammonium sulfate. Mean analytical recovery for this extraction process exceeded that with diethyl ether alone: 91.5% (n = 5).

Parallelism of dilution of neonatal serum by the DPC kit. Neonatal and infant serum (group C) was diluted with DPC zero calibrator and 25-μL portions of the dilutions were assayed with the DPC kit. The results (Table 3) for various dilutions of neonatal serum pool did not parallel those for the 17-OHP standard.

Effect of ultrafiltration. The concentration in the pooled specimen was 37.5 nmol/L before ultrafiltration (3.5 nmol/L was propanol/heptane extractable). The ultrafiltrate contained 0.8 nmol/L, the non-ultrafiltrable residual protein-rich material contained >60.5 nmol/L. Evidently the interfering substances are not ultrafiltrable.

Correlation of 17-OHP with dehydroepiandrosterone sulfate. Because the immunoreactive material in neonates’ and infants’ plasma was very polar and was extractable by solvents that are effective against steroid sulfates, we assayed plasma from 21 individual patients in group C for both dehydroepiandrosterone sulfate (17) and 17-OHP by the DPC method. There was no correlation between these (r = 0.384, P > 0.10), showing that dehydroepiandrosterone sulfate is not the steroid causing most of the interference.

Effect of glucuronidase and sulfatase on the material immunoreactive by the DPC kit. The results are shown in Table 4.

Experience with the DPC Neonatal 17-OHP Kit for Blood Spots

Blood spots prepared from 35 neonates and infants gave a non-gaussian distribution with a mean (and SD) of 45.0 (41.0) nmol/L, a median of 32.7 nmol/L, and a range of 1.2–140.7 nmol/L. All were within the range of normal given by the manufacturer (<19.4 nmol/L for term and <194 nmol/L for premature babies, calculated as concentration in serum from the blood spots).

Discussion

The DPC Coat-A-Count direct assay for 17-OHP is convenient and simple to perform. The use of an 125I tracer and absence of an extraction step significantly shorten technologist time required to complete a batch of assays as compared with our in-house assay. Although the DPC assay is a coated-tube methodology, assay precision (Table 1) is better than that of our in-house assay (CV 15.5% at 7.1 nmol/L, 10.5% at 18.6 nmol/L). The correlation with our in-house assay was adequate (Figure 1). The difference between the methods probably reflected the poorer precision of our in-house assay, differences in antibody specificity, and differences in the steroid matrix presented to the two antibodies because of inclusion or omission of the extraction step.

A group of young adults and children older than six

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**Table 3. Parallelism of Dilution of 17-OHP Concentration from Pooled Infant Plasma as Measured by the DPC Method**

<table>
<thead>
<tr>
<th>Dilution*</th>
<th>Observed</th>
<th>Expected</th>
<th>Recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>58.6</td>
<td>58.6</td>
<td>100</td>
</tr>
<tr>
<td>2 in 3</td>
<td>34.9</td>
<td>39.1</td>
<td>93.3</td>
</tr>
<tr>
<td>1 in 2</td>
<td>22.7</td>
<td>29.3</td>
<td>77.5</td>
</tr>
<tr>
<td>1 in 3</td>
<td>11.3</td>
<td>19.5</td>
<td>57.9</td>
</tr>
<tr>
<td>1 in 5</td>
<td>5.2</td>
<td>11.7</td>
<td>44.4</td>
</tr>
<tr>
<td>1 in 10</td>
<td>2.3</td>
<td>5.9</td>
<td>39.0</td>
</tr>
<tr>
<td>1 in 20</td>
<td>0.7</td>
<td>2.9</td>
<td>24.1</td>
</tr>
</tbody>
</table>

*With DPC zero calibrator.

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**Table 4. Effect of Glucuronidase and Sulfatase on the Substances in Neonatal Serum Measured as 17-OHP (nmol/L) by the DPC Method, and Their Extractability with Solvent Systems**

<table>
<thead>
<tr>
<th>Extraction solvent</th>
<th>Propanol/</th>
<th>Diethyl ether</th>
<th>Ammonium sulfate/ diethyl ether</th>
</tr>
</thead>
<tbody>
<tr>
<td>No extraction</td>
<td>5.3</td>
<td>4.7</td>
<td>36.6</td>
</tr>
<tr>
<td>Pooled neonatal serum</td>
<td>41.0</td>
<td>13.1</td>
<td>19.5</td>
</tr>
<tr>
<td>Pooled neonatal serum treated with β-glucuronidase and sulfatase</td>
<td>58.5</td>
<td>38.8</td>
<td>21.2</td>
</tr>
</tbody>
</table>
months (group B) had values for serum 17-OHP as measured by the DPC assay that were similar to those reported by the manufacturer for adults. The 97.5th centile of the log distribution for the children was 6.3 nmol/L. This value included one five-year-old child of Jewish extraction who appeared to have a value marginally higher than his peers (13.6 nmol/L), who was examined and found to be normal on physical examination by pediatric endocrinologists, but who could have been heterozygous for non-classical CAH in view of his ethnic background (16). In contrast, most values for samples from children younger than three months exceeded 6.3 nmol/L, many having 17-OHP concentrations that were more than 10-fold higher. These concentrations were unexpected, because our in-house assay had not given high values as frequently in this group. Because of this, we assessed the effect of preceding the DPC assay with an extraction procedure. As we have shown, much of the substance (or substances) in neonates' serum that is immunoreactive with the DPC direct assay is not extractable from serum by solvents that effectively extract 17-OHP and many other steroids. The most effective combination of solvents tested was diethyl ether/ethanol with added ammonium sulfate. This combination is effective in solubilizing steroid sulfates (19), so some of the materials could be steroid sulfates, but we were unable to decrease assay interference by incubating sera with sulfatase/glucuronidase preparations (Table 4). On the contrary, some additional immunoreactive material was released by such incubation. This material appeared to be more readily extractable than the material present in the original serum. The bulk of the original immunoreactive material was not extractable with dichloromethane, propanol/heptane, or diethyl ether—and enzyme treatment did not change this. Much of the immunoreactive material was extractable with diethyl ether/ethanol/ammonium sulfate, and extractability into this solvent system was not affected by enzyme treatment. The immunoreactive materials in neonatal sera did not show parallelism to 17-OHP on dilution (Table 3). They remained with the macromolecular fraction of serum on ultrafiltration, suggesting that they may be macromolecular or, perhaps more likely, that they are protein-bound. They are unlikely to be protein, because some were extractable by ether/ethanol after treatment with ammonium sulfate.

A direct assay kit for 17-OHP from a second company (RSI/Immchem, Carson, CA 90746) was also sensitive to similar interference by substances in neonates' sera (data not shown), and recent kit inserts have warned of this problem.

A review of the literature on expected values for serum 17-OHP suggests that methodological problems associated with the different steroid matrix in samples from neonates have led to some confusion. There is general agreement that 17-OHP (like progesterone) increases during pregnancy in maternal and fetal blood, resulting in 17-OHP concentrations in cord serum that are much higher than those of older children or non-pregnant adults. After birth, values declined rapidly to reach those of the normal adult by two to seven days in the studies of 60 term infants (20 and 27) normal newborns (21). Premature and sick term infants, between days 2 and 5 after birth, have two- to threefold higher values than do normal infants of the same age (22). The increased 17-OHP probably reflects the stress-related increase in corticotropin production. The magnitude of the increase probably depends on the severity of the illness, but it is also influenced by the analytical method used and whether or not an extraction procedure is used (15). Godé et al. (22) found that premature babies had mean 17-OHP concentrations about two- to threefold those of term infants, but Hughes et al. (23) described increases of approximately six- to eightfold with an assay that had 7% cross-reactivity with 17α-hydroxyprogrenolone. 17α-Hydroxyprogrenolone is less likely to be a problem with the DPC assay, because the cross-reaction is only 0.4%. We saw no relationship between 17-OHP and prematurity or chronological age (Figure 3). Moreover, when we extracted sera from Group A, many of whom were sick premature infants, and analyzed the reconstituted residue from the extract in the DPC assay, we found that in most cases the 17-OHP concentration was about the same as that for older children and adults. The concentrations of many steroids in neonates' serum (and also the concentrations of their metabolites in urine) are quite different from those for older children and adults (21-27). The fetal zone of the adrenal involutes during the first few postnatal weeks, and the adult zone doubles its volume in the first three postnatal months. During this time there is a decline in the concentrations in serum of the steroids that characterize the fetal zone with its relative deficiency of 3β-hydroxysteroid dehydrogenase. For example, the mean (and SD) concentrations of pregnenolone sulfate were 1371 (816) nmol/L two to 10 days after birth, 972 (638) at 31–60 days, and 43 (41) at one to two years, and the concentration only increases to 133 (65) nmol/L in adults (26). In addition to pregnenolone sulfate, 16α-hydroxydehydroepiandrosterone, pregnenolone, and several other steroids are all present in greatly increased concentration in neonatal serum (21, 24). When the concentrations of these substances are so much higher than those of 17-OHP they could probably begin to affect even assays in which highly specific antibodies are used.

In several neonatal screening programs in which the direct assay of 17-OHP eluted from filter paper was used, it also was observed that there was some interference by water-soluble substances (15, 28). Wallace et al. (15) increased specificity by including an extraction step with diethyl ether. The substances responsible for the interference have not been characterized in serum, but they correlate with several urinary 3β-hydroxy-5-ene steroid metabolites in urine (27). They seem to affect results of methods in which antibodies generated against 17-OHP linked at its 7-position to its antigen (DPC and Immchem kits) are used, as shown in the present study, and also those in which antibodies produced against 17-OHP linked to its antigen via the 3- position (15) are used, even though linkage through the 3- position is said to provide more specific antibodies (10, 29).

Interference has previously been observed with some other kits for direct assay of steroid hormones. Reports of problems with measurement of cortisol in urine (30) or in plasma in chronic renal failure (31), testosterone (32), and estradiol in neonatal serum (33), and our own studies with 17-OHP all emphasize the need for thorough clinical evaluation of such products before general marketing and the inclusion of recommendations for an appropriate confirmatory extraction procedure where results are not consistent with the patient's clinical condition. In the case of the DPC 17-OHP kit, we recommend that it be used only with patients older than six months unless an extraction step is added, and that laboratories using this kit pay special attention to determining the age of any patient for whom an increased result is obtained, before the result is reported.
We thank Mr. Dan Lichtman of Intermedico, Markham, ON; Dr. Peter Bodlaender of Diagnostic Products Corp., Los Angeles, CA; and Dr. Robert Hudak of RSL/Immuchem, Carson, CA, for providing kits and for helpful discussions.

References