

## Congenital Adrenal Hyperplasia

### Laboratory support of diagnosis and management

#### Clinical Background

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders caused by deficiency in one or more of the enzymes required for synthesis of cortisol, aldosterone, and sex steroids in the adrenal gland. In most instances, the levels of steroids proximal to the enzyme defect (precursors) are elevated, and the levels of those distal to the defect (products) are decreased (Figure). Decreased cortisol production leads to an increase of adrenocorticotrophic hormone (ACTH), and the resulting adrenal stimulation leads to a further increase of the steroids, and their associated metabolites, proximal to the defect. Shunting of precursor steroids may then result in increased production of sex hormones. Additionally, decreased production of aldosterone can lead to renal salt loss and hypotension. Genes coding for the enzymes have been identified (Table 1) and nomenclature reflects these findings, eg, 21-hydroxylase deficiency is coded for by *CYP21A2* and the defect is frequently referred to by the gene name.<sup>1,2</sup>

The clinical manifestations of CAH vary with the enzyme defect present (Table 1) and the degree of deficiency. While individuals with nonclassic disease (ie, partial deficiency) may be asymptomatic, those with severe disease may exhibit marked adrenal insufficiency and salt-wasting, genital ambiguity, and virilization ranging from mild to complete masculinization of the external genitalia of female (XX) fetuses.<sup>3</sup> Masculinization of females can be prevented by maternal treatment with dexamethasone beginning before the 7<sup>th</sup> week of gestation in at-risk pregnancies.<sup>4</sup> Treatment is then discontinued if DNA testing indicates a low likelihood of CAH or male (XY) genotype is established.

Neonatal screening for 21-hydroxylase deficiency (*CYP21A2*), the most common cause of CAH, is performed routinely in many states by measuring 17-hydroxyprogesterone (17-OHP) in filter paper blood samples obtained from newborns. Diagnosis in infants with an elevated 17-OHP or those with clinical manifestations of CAH is accomplished by

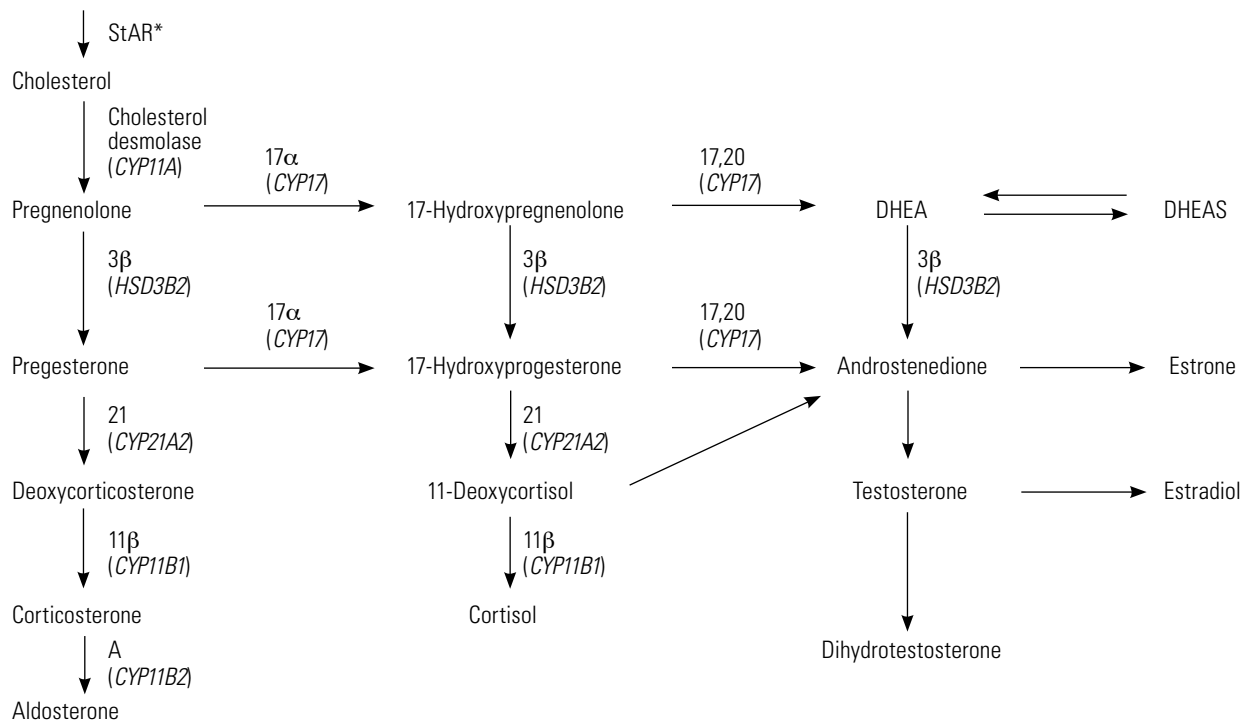


Figure. Synthetic pathways of adrenal steroid synthesis. Enzymes and encoding genes are indicated at arrows. When an enzymatic defect occurs, steroids proximal to the defect increase and are frequently shunted into other products, and steroids distal to the defect decrease (eg, a deficiency of 11 $\beta$ -hydroxylase will cause increased levels of deoxycorticosterone, progesterone, 11-deoxycortisol, and 17-hydroxyprogesterone and decreased levels of corticosterone, aldosterone, and cortisol). 17 $\alpha$ , 17 $\alpha$ -hydroxylase; 17,20, 17,20-lyase; 3 $\beta$ , 3 $\beta$ -hydroxysteroid dehydrogenase; 21, 21-hydroxylase; 11 $\beta$ , 11 $\beta$ -hydroxylase; A, two-step process of aldosterone synthesis: 1) hydroxylation of corticosterone to form 18-hydroxycorticosterone, 2) oxidation of 18-hydroxycorticosterone to form aldosterone; DHEA, dihydroepiandrosterone; DHEAS, dihydroepiandrosterone sulfate.

\*StAR, steroid acute regulatory protein: transports cholesterol from the outer to inner mitochondrial membrane

measuring blood and/or urine steroid and metabolite levels. Additionally, DNA analysis can identify *CYP21A2* mutations, which is helpful for determining carrier status, confirming the diagnosis in affected individuals, and establishing the diagnosis prenatally.

### 21-Hydroxylase Deficiency

21-Hydroxylase deficiency (*CYP21A2*) accounts for 90% to 95% of CAH cases, and classic and nonclassic forms have been described.<sup>5</sup> Classic 21-hydroxylase deficiency has an incidence of 1:5,000 to 1:15,000 live births in Western populations, though higher frequencies have been identified in certain ethnic groups.<sup>5</sup> The disorder is characterized by markedly diminished or absent 21-hydroxylase activity, which results in decreased levels of deoxycorticosterone, 11-deoxycortisol, corticosterone, cortisol, and aldosterone and increased levels of 17-OHP and androstenedione. Affected individuals typically present at birth or in the neonatal period with either a virilizing or salt-wasting form. Female (XX) infants with virilizing 21-hydroxylase deficiency have varying degrees of masculinization ranging from clitoral

enlargement to complete development of male external genitalia, which may lead to inaccurate sex assignment at birth. Male infants (XY) have normal male genitalia. Approximately three-quarters of affected infants also have mineralocorticoid deficiency that leads to salt-wasting. Symptoms of hyponatremia, hyperkalemia, volume depletion, and decreased blood pressure generally appear within the first 2 weeks of life.

Nonclassic 21-hydroxylase deficiency is thought to have an incidence as high as 1:1,000 and is characterized by marginally decreased 21-hydroxylase activity.<sup>5</sup> Affected individuals typically do not have developmental abnormalities or salt-wasting. Presentation is in childhood or post puberty with evidence of androgen excess. In boys, increased androgens typically manifest as sexual precocity, whereas in girls increased pubic hair growth and/or clitoral enlargement is seen. In women, nonclassic 21-hydroxylase deficiency is frequently confused with polycystic ovary syndrome.

The genetic diagnosis of 21-hydroxylase deficiency is complex because of the large number of unique mutations

Table 1. Characteristics of Congenital Adrenal Hyperplasia Enzyme Deficiencies

	21-Hydroxylase		11 $\beta$ -Hydroxylase	17 $\alpha$ -Hydroxylase	3 $\beta$ -Hydroxysteroid Dehydrogenase	Aldosterone Synthase	Steroid Acute Regulatory Protein (StAR)	P450 Oxidoreductase
	Classic	Nonclassic						
Gene	<i>CYP21A2</i>	<i>CYP21A2</i>	<i>CYP11B1</i>	<i>CYP17</i>	<i>HSD3B2</i>	<i>CYP11B2</i>	<i>STAR</i>	<i>CYPOR</i>
Incidence*	1:5,000-15,000	1:1000	1:100,000	Rare	Rare	Rare	Rare	Rare
Elevated Steroids	17-OHP androstenedione	17-OHP (post ACTH) androstenedione	DOC 11-deoxycortisol	DOC corticosterone progesterone	DHEA 17-OH pregnenolone pregnenolone	Corticosterone 18-OHC (type II)	None	Pregnenolone progesterone DOC
Decreased Steroids	Aldosterone corticosterone (salt-wasting) cortisol (simple virilizing)	None	Cortisol corticosterone aldosterone (classic)	Cortisol aldosterone 17-OHP	Cortisol aldosterone	Aldosterone 18-OHC (type I)	All	Cortisol ( $\downarrow$ ACTH response)
Age at Diagnosis	Infancy	Childhood/puberty	Neonatal to adult	Puberty	Early infancy (severe) post puberty (mild)	Neonatal	Neonatal	Infancy/childhood
Genitalia Females (XX)	Virilized	$\pm$ Mild virilization	Mild-severe virilization	No puberty	Mild virilization	Normal	No puberty	Ambiguous
Males (XY)	Normal	Normal	Normal	Ambiguous	Ambiguous	Normal	Ambiguous	Ambiguous
Androgens	$\uparrow$	$\uparrow$	$\uparrow$	$\downarrow$	$\downarrow$ in males $\uparrow$ in females	Normal	$\downarrow$	$\downarrow$
Estrogens	$\downarrow$	$\downarrow$	$\downarrow$ in females	$\downarrow$	$\downarrow$	Normal	$\downarrow$	$\downarrow$
Na <sup>+</sup>	$\downarrow$ in salt-wasting	Normal	$\uparrow$	$\uparrow$	$\downarrow$	$\downarrow$	$\downarrow$	Normal
K <sup>+</sup>	$\uparrow$	Normal	$\downarrow$	$\downarrow$	$\uparrow$	$\uparrow$	$\uparrow$	Normal
Blood Pressure	$\downarrow$	Normal	$\uparrow$	$\uparrow$	$\downarrow$	$\downarrow$	$\downarrow$	Normal

17-OHP, 17-hydroxyprogesterone; ACTH, adrenocorticotropic hormone; DOC, deoxycorticosterone; DHEA, dihydroepiandrosterone; 18-OHC, 18-hydroxycorticosterone; Na<sup>+</sup>, sodium; K<sup>+</sup>, potassium.

\*Incidence found in the general population. Some disorders have a higher incidence in certain ethnic groups.<sup>1,5</sup>

that may result in decreased enzyme activity and interactions between *CYP21A2* and its pseudogene *CYP21A*. The majority of mutations arise from 2 types of recombination between *CYP21A2* and *CYP21A*: 1) deleterious mutations that have been transferred from the pseudogene to *CYP21A2* during mitosis (75% of cases), and 2) unequal recombinations between the gene and pseudogene that result in deletion of the intervening 30-kb segment (20% of cases).<sup>5</sup> Although over 60 relevant mutations have been identified, 11 account for approximately 90% of those found in heterozygous carriers.<sup>5</sup> Additionally, in affected individuals, 1% to 2% of abnormal alleles contain spontaneous mutations that are not carried by either parent. Enzyme activity varies with the mutations present, and 3 phenotypic categories, which manifest 0%, 1% to 2%, or 20% to 60% of normal activity, have been identified.<sup>5</sup> Although allelic variation accounts for 80% to 90% of the phenotypic variation, the affected individual's unique genetic factors (eg, steroid receptor binding properties) may influence final expression.<sup>5</sup>

### 11 $\beta$ -Hydroxylase Deficiency

11 $\beta$ -Hydroxylase deficiency (*CYP11B1*) accounts for 5% to 8% of CAH cases.<sup>6</sup> Deficiency of 11 $\beta$ -hydroxylase leads to decreased levels of cortisol, corticosterone, and aldosterone and increased levels of deoxycorticosterone and 11-deoxycortisol. Salt-wasting does not occur as with 21-hydroxylase deficiency; however, virilization of female (XX) fetuses can be as severe. Though a small percentage of affected individuals present with salt-wasting, elevated blood pressure manifests early in life in approximately two-thirds of patients and, along with elevated deoxycorticosterone and 11-deoxycortisol levels, clinically distinguishes 11 $\beta$ - from 21-hydroxylase deficiency (Table 1). A late-onset form that typically presents with signs of androgen excess is analogous to nonclassic 21-hydroxylase deficiency.

### 17 $\alpha$ -Hydroxylase Deficiency

17 $\alpha$ -Hydroxylase deficiency (*CYP17*) accounts for approximately 1% of all CAH cases with an estimated incidence of 1:50,000 newborns.<sup>7</sup> The *CYP17* gene encodes an enzyme that catalyzes both 17 $\alpha$ -hydroxylation and 17,20-lyase reactions. Isolated deficiency of either activity has been reported; however, a combined deficiency in which there is failure of catalysis of both reactions is the most common form. Affected individuals have decreased levels of cortisol, androgens, and estrogens. Presentation is typically at puberty; females (XX) have primary amenorrhea and lack secondary sexual characteristics, and males (XY) are found to have complete pseudohermaphroditism (ie, female external genitalia, absence of uterus and fallopian tubes, and intra-abdominal testes). At the time of diagnosis, individuals are usually found to be hypertensive and hypokalemic.

### 3 $\beta$ -Hydroxysteroid Dehydrogenase Deficiency

3 $\beta$ -Hydroxysteroid dehydrogenase deficiency (*HSD3B2*) is a rare form of CAH characterized by increased levels of pregnenolone, 17-hydroxypregnenolone, and DHEA and decreased levels of all other adrenal steroids.<sup>8</sup> Affected individuals usually present in infancy with signs of adrenal insufficiency. Female (XX) infants will typically have mild virilization. Phenotypic variation in male (XY)

infants may range from hypospadias to complete male pseudohermaphroditism.

### Aldosterone Synthase Deficiency

Aldosterone synthase deficiency (*CYP11B2*) is a rare form of CAH in which only aldosterone synthesis is affected. Two forms have been identified; type I is characterized by decreased, and type II by increased, 18-hydroxycorticosterone.<sup>9</sup> Infants usually present within the first 5 days of life with failure to thrive, recurrent dehydration secondary to salt-wasting, decreased blood pressure, and acidosis.

### Steroid Acute Regulatory Protein (StAR) Deficiency

StAR deficiency is responsible for congenital lipid adrenal hyperplasia, a defect of cholesterol transport resulting in deficiency of all adrenal steroids. It is the rarest of the congenital adrenal steroid defects and has been fatal in two-thirds of the reported cases. The defect was thought to reside in *CYP11A*, the gene that codes for the cholesterol side-chain cleavage enzyme; however, recent molecular studies suggest the defect resides on chromosome 8 in the *STAR* gene which encodes a phosphoprotein that enhances cholesterol transport from the outer to inner mitochondrial membrane.<sup>10</sup> Affected individuals present in the neonatal period with severe adrenal insufficiency manifested by failure to thrive, vomiting, diarrhea, hyponatremia, and hypokalemia. Males (XY) typically have normal female external genitalia.

### P450 Oxidoreductase Deficiency (ORD)

ORD (*CYPOR*) is a newly identified cause of CAH.<sup>11,12</sup> P450 oxidoreductase contributes electrons to microsomal P450 enzymes, and its deficiency results in decreased 17 $\alpha$ - and 21-hydroxylase activity. Steroid profiles may show increased levels of 17-OHP, 21-deoxycortisol, progesterone, and pregnenolone. Baseline cortisol is typically normal; however, the response to ACTH is decreased. Genital ambiguity is frequently seen, and the majority of patients have Antley-Bixler syndrome, a unique constellation of craniofacial and skeletal abnormalities. ORD is also considered a potential cause of decreased estriol in mid-trimester Down syndrome screening tests.

### Individuals Suitable for Testing

- Newborns with a positive CAH screening test
- Newborns with ambiguous genitalia
- Newborns and infants with evidence of adrenal insufficiency and/or unexplained sodium and potassium abnormalities
- Infants or children with evidence of CAH not attributable to known causes or in whom Antley-Bixler syndrome is suspected
- Children with evidence of precocious or delayed puberty or unexplained hypertension
- Women with polycystic ovary syndrome, hirsutism, and/or evidence of estrogen deficit
- Individuals with suspected androgen excess
- Individuals with a family history of CAH, and their partners, who desire carrier screening
- Pregnant women at risk for a fetus affected with *CYP21A2* mutations who desire prenatal diagnosis

## Test Availability

### Steroid Assays

The diagnosis of enzymatic defects responsible for CAH relies upon accurate measurement of steroid and steroid metabolite levels and the calculation of precursor-product ratios. Quest Diagnostics Nichols Institute utilizes mass spectrometry technology for increased sensitivity and discrimination in steroid measurement. While assays that measure steroid and metabolite levels in blood or urine may be ordered individually, CAH panels that include the analytes and corresponding ratios necessary for diagnosis are available (Table 2). Additionally, plasma renin activity (PRA) and direct renin assays are available for use in monitoring treatment of individuals with salt-wasting.

### DNA Analysis

#### CAH (21-Hydroxylase Deficiency) Common Mutations

Using 4 polymerase chain reactions to examine the gene, pseudogene, and recombinant genes, this test detects the most common mutations of *CYP21A2*: P30L, In2G, G110del8, I172N, exon 6 cluster mutation (1235N, V236E, M238K), V281L, F306+1nt, Q318X, R356W, P453S, and 30-kb deletion. These 12 mutations and 30-kb deletion are responsible for approximately 90% of the *CYP21A2* defects that can result in 21-hydroxylase deficiency.

#### CAH (21-Hydroxylase Deficiency) Rare Mutations

This test provides complete sequencing of *CYP21A2*.

### Chromosome Analysis

Chromosome analysis differentiates XX and XY genotypes in those presenting with ambiguous genitalia.

## Test Selection

### Steroid Assays

Blockage of an adrenal biosynthetic pathway due to an enzymatic defect results in increased levels of the enzyme's precursors and associated metabolites as well as increased precursor-product ratios. In severe 21- and 11 $\beta$ -hydroxylase and 3 $\beta$ -hydroxysteroid dehydrogenase deficiencies, baseline steroid and precursor-product ratios are frequently adequate for diagnosis; however, steroid measurement after ACTH stimulation is usually necessary for diagnosis when partial deficiency is suspected. ACTH stimulates adrenal hormone synthesis, which accentuates precursor steroid levels and diagnostic ratios. For example, ACTH stimulation followed by 17-OHP measurement is useful for evaluating newborns with increased 17-OHP levels in the absence of virilization or adrenal insufficiency.

In newborns with suspected CAH (ie, adrenal insufficiency, virilization, 17-OHP >400 ng/dL, family history), assay selection must be guided by clinical findings and other laboratory information (Table 1). The differential diagnosis of virilizing CAH in a newborn includes 21-hydroxylase, 11 $\beta$ -hydroxylase, and 3 $\beta$ -hydroxysteroid dehydrogenase deficiencies as well as ORD. In those with salt-wasting, aldosterone synthase and StAR deficiencies must also be considered.

Table 2 specifies the clinical use for each of the CAH panels offered by Quest Diagnostics. CAH Panel 1 enables diagnosis of the 2 most common forms of CAH: 21- and 11 $\beta$ -hydroxylase deficiencies. Panel 6b serves as a comprehensive screen that is useful when a broader differential diagnosis is being considered. During the neonatal period, CAH Panel 11 can distinguish between and diagnose 21-, 11 $\beta$ -, 17 $\alpha$ -hydroxylase, and 3 $\beta$ -hydroxysteroid dehydrogenase deficiencies.<sup>13</sup> This urine-based assay is an alternative to serum measurements when clinical suspicion for CAH is high or the 17-OHP screen is positive. Other CAH panels are used to diagnose the enzyme defects responsible for rare causes of CAH and should be selected when other laboratory and clinical findings suggest an uncommon type of CAH.

Treatment of CAH requires monitoring adrenal steroid levels to ensure correct dosing of glucocorticoid and/or mineralocorticoid replacement. CAH Panel 7 is designed for monitoring treatment of 21-hydroxylase deficiency. Plasma renin activity and direct renin assays are useful for monitoring mineralocorticoid replacement in individuals with salt-wasting.

### DNA Analysis

#### CAH (21-Hydroxylase Deficiency) Common Mutations

This test is suitable for diagnosis in infants with a positive newborn screen and in individuals known or suspected to be 21-hydroxylase deficient. It is also useful for carrier screening in those with a family history (especially first degree relatives of affected individuals) and in spouses of affected individuals or carriers (to identify high-risk pregnancies). DNA analysis is the preferred method for determining carrier status in individuals with marginally elevated 17-OHP levels, because post-ACTH stimulation 17-OHP levels overlap in normal individuals and carriers.<sup>3</sup> This assay is also suitable for prenatal diagnosis of 21-hydroxylase deficiency when performed on CVS or amniotic fluid samples.

#### CAH (21-Hydroxylase Deficiency) Rare Mutations

This test is indicated for 21-hydroxylase deficiency-affected individuals in whom only 1 or none of the common mutations have been identified and for individuals whose family history includes a rare mutation.

### Chromosome Analysis

Karyotyping is suitable to determine the genotype (XX or XY) and thereby establish gender in infants born with ambiguous genitalia.

## Test Interpretation

### Steroid Assays

An early morning 17-OHP level <200 ng/dL almost always eliminates the diagnosis of 21- or 11 $\beta$ -hydroxylase deficiency. Though 17-OHP levels up to 400 ng/dL may be normal, those >200 ng/dL require evaluation. Levels >400 ng/dL are consistent with 21- or 11 $\beta$ -hydroxylase deficiency.<sup>3,5</sup> Prematurity, illness, and stress can cause elevations in 17-OHP levels.

Elevated precursor-product ratios are diagnostic of enzyme deficiencies and are used to discriminate between causes of CAH. In normal children, adolescents, and adults, precursor-product ratios associated with the 3 $\beta$ -HSD, 21-, and 17 $\alpha$ -hydroxylase enzymes will not exceed 10, while the ratios associated with 11 $\beta$ -hydroxylase and aldosterone synthase

will not exceed 15. In unaffected individuals, precursor-product ratios are similar before and after ACTH stimulation.

After puberty, a post-ACTH stimulation 17-OHP level between 1500 ng/dL and 10,000 ng/dL is considered diagnostic of nonclassic 21-hydroxylase deficiency. An 11-deoxycortisol level >350 ng/dL or 11-deoxycortisol:cortisol ratio >15

Table 2. Congenital Adrenal Hyperplasia Diagnostic Panels\*

	Panel 1	Panel 2	Panel 3	Panel 4	Panel 6	Panel 6b†	Panel 7†	Panel 8	Panel 9
	21-OH vs 11 $\beta$ -OH Deficiency	Salt-wasting 21-OH Deficiency	Aldosterone Synthase Deficiency	Female 17 $\alpha$ -OH Deficiency	StAR Deficiency	Comprehensive Screen	Monitor 21-OH Deficiency Treatment	Male 17 $\alpha$ -OH Deficiency	3 $\beta$ -HSD Deficiency
<b>Analyte</b>									
Aldosterone		↓	↓	↓	↓			↓	
Androstenedione	↑					•	•		↓
Cortisol (total)	↓	↓		↓	↓	•		↓	↓
Corticosterone				↑				↑	
11-Deoxycortisol (Compound S)	↑ in 11 $\beta$ ↓ in 21	↓	Normal			•			
DOC						•			
DHEA					↓	•			↑
Estradiol				↓					
18-Hydroxycorticosterone			↓ in type I ↑ in type II						
17-Hydroxypregnenolone						•			↑
17-OHP	↑	↑	Normal	↓		•	•	↓	↓
Pregnenolone					↓				
Progesterone				↑		•		↑	
Testosterone (total)	↑					•	•	↓	
<b>Diagnostic Ratio</b>									
<u>11-Deoxycortisol</u> Cortisol	>9 in 11 $\beta$								
<u>DHEA</u> Androstenedione									>9
<u>18-Hydroxycorticosterone</u> Aldosterone			<10 type I >100 type II						
<u>17-Hydroxypregnenolone</u> 17-OHP									>12
<u>17-OHP</u> 11-Deoxycortisol	>3 in 21	>3	>3						
<u>Progesterone</u> DOC									
<u>Progesterone</u> 17-OHP				>4				>4	

21-OH, 21-hydroxylase; 11 $\beta$ -OH, 11 $\beta$ -hydroxylase; 17 $\alpha$ -OH, 17 $\alpha$ -hydroxylase; StAR, steroid acute regulatory protein; 3 $\beta$ -HSD, 3 $\beta$ -hydroxysteroid dehydrogenase; DOC, deoxycorticosterone; DHEA, dihydroepiandrosterone; 17-OHP, 17-hydroxyprogesterone.

\*Analytes and expected result if enzyme defect is present are indicated for each panel.

†Results for panels 6b and 7 will be within reference range if no defect is present (6b) or treatment is adequate (7).

Not shown: Panel 11 (Tables 6 and 7) is a comprehensive neonatal profile (urine) that includes 15 steroid analytes and 11 ratios for differential diagnosis of 21-hydroxylase, 11-hydroxylase, 17 $\alpha$ -hydroxylase, and 3 $\beta$ -hydroxysteroid dehydrogenase deficiencies.

is diagnostic of 11 $\beta$ -hydroxylase deficiency. After ACTH stimulation, a 17-hydroxypregnenolone level >1500 ng/dL and 17-hydroxypregnenolone:17-OHP and DHEA:androstenedione ratios >10 are considered diagnostic of 3 $\beta$ -HSD. However, recent studies utilizing DNA analysis of *HSD3B2* to confirm disease state have questioned these criteria, and new diagnostic standards based upon 17-hydroxypregnenolone levels and 17-hydroxypregnenolone:cortisol ratios before and after ACTH stimulation have been proposed.<sup>14,15</sup>

Table 3 contains pre- and post-ACTH stimulation 17-OHP reference ranges for infants, children, and adults. Table 4 contains additional steroid reference ranges for infants and children pre- and post-ACTH stimulation, and Table 5 contains infant and children reference ranges for precursor-product ratios pre- and post-ACTH stimulation. Tables 6 and 7 contain reference ranges for diagnostic ratios and steroids, respectively, for CAH Panel 11 (neonatal urine panel).

Steroid and metabolite levels and ratios should be interpreted in conjunction with other laboratory and clinical findings.

### DNA Analysis

The following information will assist in understanding test results. A complete family history and parental and affected sibling genotypes are required for the most accurate interpretation of 21-hydroxylase deficiency DNA testing. Assistance is available from our Genetic Counselors by calling 1-866-GENE-INFO (1-866-436-3463).

### CAH (21-Hydroxylase Deficiency) Common Mutations

A negative result indicates none of the 12 common mutations or 30-kb deletion responsible for 21-hydroxylase deficiency were detected. In an individual without clinical symptoms of CAH, this lowers, but does not eliminate the risk of a mutation being present; the residual risk of being a carrier depends upon the individual's family history. In an individual affected with CAH, a negative result suggests the presence of a rare mutation or a cause other than 21-hydroxylase deficiency.

Three different positive results may be reported:

1. Positive: 1 copy of *CYP21A2* contains at least 1 common mutation, while the other copy does not contain any of the 12 common mutations or 30-kb deletion. In most instances the individual is considered a carrier and may exhibit mild symptoms depending upon the mutation present. DNA testing of parents and affected siblings may be necessary to eliminate the possibility of gene duplication (the individual may have a functional gene on one chromosome, and a functional as well as a mutated gene on the second chromosome). Unrecognized, the individual may be incorrectly classified as a carrier.
2. Positive: both copies of *CYP21A2* contain common mutations (ie, 2 distinct copies of *CYP21A2* are identified and both contain 1 or more of the 12 common mutations or 30-kb deletion). Individuals will be affected with 21-hydroxylase deficiency, the severity determined by the mutations present.

Table 3. 17-Hydroxyprogesterone Reference Ranges Pre- and Post-ACTH Stimulation

	Baseline (ng/dL)	60 Minutes Post ACTH Stimulation (ng/dL)
Males*		
18-30 y	32-307	42-250
31-40 y	42-196	42-250
41-50 y	33-195	42-250
51-60 y	37-129	42-250
Females*		
Follicular Phase	≤185	42-250
Mid-cycle Phase	≤225	
Luteal Phase	≤285	
Postmenopausal Phase	≤45	
Children†		
Premature Infants (31-35 wk)	≤360	
Term Infants (birth-5 d)	≤420	
1-20 mo	11-170	85-465
1-5 y	4-115	50-350
6-12 y	7-69	75-220
Tanner Stage II-III		
Males	12-130	69-310
Females	18-220	80-420
Tanner Stage IV-V		
Males	51-190	105-230
Females	36-200	80-225

\*Includes data from references 16-19.

†Includes data from references 20-22.

3. Positive for the heterozygous presence of at least 2 common mutations (ie, mutations are present, but it cannot be determined if they are on the same or separate chromosomes). If the mutations all reside on the same chromosome (*cis* configuration), the individual is a carrier; if they are on separate chromosomes (*trans* configuration), the individual will be affected with 21-hydroxylase deficiency. DNA testing of parents and affected siblings is frequently necessary to determine if the mutations are in *cis* or *trans* configuration.

Mutations will be identified in approximately 90% of carriers; the other 10% may have a rare mutation not tested for in this assay. Furthermore, this test will detect both relevant mutations in approximately 81% of affected individuals, only 1 relevant mutation in another 18%, and no mutations in the final 1% of affected individuals. Thus, rare mutation analysis may be needed to detect additional mutations in affected individuals.

Table 4. Observed Ranges for Serum Adrenal Steroids in Infants and Children. Values Before (B), After (A), and Response ( $\Delta$ ) to Rapid ACTH Test

Steroid		26-28 wk*	34-36 wk*	1-6 mo	<1 y	1-5 y	6-12 y	Pubertal Male	Pubertal Female
Pregnenolone	B	260-2100	203-1024	10-150	10-137	10-48	15-45	15-84	24-50
	A	962-3179	637-1888	110-359	49-359	34-135	38-104	33-218	37-149
	$\Delta$	70-2673	162-1685	20-282	19-282	4-114	16-73	6-193	9-101
Progesterone	B	18-640	-	5-53	5-80	8-64	5-93	6-1286	17-145
	A	52-1348	-	74-200	74-200	51-233	38-204	32-1069	35-223
	$\Delta$	29-796	-	35-165	35-192	19-192	22-170	0-104	0-192
17-OH pregnenolone	B	375-3559	559-2906	52-828	13-788	9-98	10-177	19-346	50-516
	A	2331-11440	831-9760	633-3286	373-3125	43-702	67-624	84-817	239-1525
	$\Delta$	1219-9799	346-8911	229-3104	200-3000	15-680	60-500	65-750	108-1280
17-OH progesterone	B	124-841	186-472	13-173	11-173	4-114	7-69	12-190	18-220
	A	285-1310	334-1725	85-250	85-466	50-350	75-218	69-313	80-422
	$\Delta$	50-596	18-1253	52-193	50-275	30-300	50-250	7-281	9-287
DHEA	B	236-3640	223-3640	26-505	26-500	9-42	11-153	25-400	69-686
	A	1320-8952	727-7821	67-1453	18-1100	21-98	34-322	62-509	95-1557
	$\Delta$	408-8610	32-7219	28-1343	5-600	5-70	20-220	22-386	26-1233
Androstenedione	B	92-892	90-837	6-78	6-78	5-51	7-68	17-151	43-221
	A	145-1248	183-1367	21-114	21-139	12-68	12-98	2-215	58-319
	$\Delta$	40-718	13-1084	9-76	10-75	5-60	5-60	8-121	9-118
11-Deoxycortisol	B	110-1376	70-455	10-200	10-200	7-210	14-136	11-151	15-130
	A	206-2504	81-645	101-392	80-390	98-360	95-322	87-283	78-250
	$\Delta$	15-1128	40-190	5-366	5-350	50-280	30-180	35-241	34-233
Cortisol	B	1-11	3-34	3-22	3-23	5-25	5-23	4-15	4-16
	A	6-52	16-76	27-50	32-60	22-40	17-40	15-45	16-35
	$\Delta$	4-41	6-44	19-41	17-40	5-25	5-20	5-32	7-26
Deoxycorticosterone	B	20-105	28-78	7-48	7-57	4-49	5-34	2-12	4-30
	A	44-320	28-95	40-158	20-157	26-143	19-138	13-63	12-74
	$\Delta$	17-215	1-67	13-144	26-110	23-135	16-130	10-53	7-43
Corticosterone	B	235-1108	201-5030	78-2500	78-1750	120-2030	155-1365	111-598	115-1219
	A	1667-8251	2240-11900	2225-4974	2225-6505	2150-7540	1775-7500	1723-5100	1472-5060
	$\Delta$	1338-8016	2039-10141	1149-4789	1140-5120	960-7300	1490-7300	1380-4700	1003-4740
18-OH corticosterone	B	10-670	38-779	5-300	5-310	7-155	10-74	11-82	5-73
	A	35-1500	152-2183	130-465	67-470	49-370	79-360	69-322	73-1472
	$\Delta$	16-830	114-2183	21-394	22-395	33-333	69-310	58-254	22-1467
Aldosterone	B	5-635	12-736	2-71	2-130	2-37	3-21	2-32	1-14
	A	13-1046	42-1365	5-166	5-167	13-85	14-50	10-34	10-33
	$\Delta$	8-517	28-629	3-123	4-122	7-54	4-40	0-22	7-25

Results in ng/dL except cortisol ( $\mu$ g/dL); ACTH 1-24 (250  $\mu$ g) given as intravenous bolus; data from extraction, chromatography, RIA; References 21-23.

\*Premature. Samples obtained on postnatal days 2-4.

### CAH (21-Hydroxylase Deficiency) Rare Mutations

Negative results reduce, but do not eliminate the possibility that an unaffected individual is a carrier, because certain sequence alterations (eg, large deletions) may not be detected. The residual risk is influenced by the individual's family history.

Positive results include: 1) sequence alterations known to be pathogenic, 2) sequence alterations predicted to be pathogenic but not reported in literature, 3) sequence alterations known to be benign, 4) sequence alterations predicted to be benign, and 5) sequence alterations of unknown clinical significance. In the presence of positive clinical findings, the detection of 2 mutations known or predicted to be pathogenic, or 1 in addition to a previously identified mutation, is consistent with a diagnosis of

21-hydroxylase deficiency. The detection of 2 mutations known or predicted to be pathogenic in the absence of clinical symptoms of CAH suggests *cis* configuration of the mutations.

Results must be interpreted in light of the individual's clinical status, and family history including genotypes of parents and siblings.

### Ordering Information

Early morning samples are preferred for steroid assays. Specify age, sex, and suspected clinical diagnosis on the test request form. Refer to the Quest Diagnostics Directory of Services for specific test codes, CPT codes, and specimen collection and handling requirements.

Table 5. Normal Adrenal Enzyme Precursor/Product Ratio Ranges in Infants and Children. Values Before (B) and After (A) Rapid ACTH Test\*

Precursor	26-28 wk		34-36 wk		1-6 mo		6 mo – 1 y		1-5 y		6-12 y		Pubertal	
Product Ratios	B	A	B	A	B	A	B	A	B	A	B	A	B	A
<b>21-OH Deficiency</b>														
<u>Progesterone</u>														
Deoxycorticosterone	1.6-9.4	1.1-9.8	–	–	0.3-7.0	0.9-4.0	0.3-7.0	0.9-4.0	0.5-10	0.6-6.0	0.9-8.4	0.9-3.7	1.3-14	1.2-6.6
<u>17-OH progesterone</u>														
11-Deoxycortisol	0.4-2.4	0.3-2.1	0.9-4.8	0.8-4.2	0.4-3.1	0.5-2.0	0.4-3.1	0.5-2.0	0.3-2.1	0.5-1.6	0.2-2.1	0.5-1.6	0.2-3.7	0.4-2.7
<b>11β-OH Deficiency</b>														
<u>11-Deoxycortisol</u>														
Cortisol	25-300	10-189	3-115	3-26	0.8-10	2.4-1.0	0.8-10	2.4-10	1.0-6.8	3.8-11	1.2-9.0	2.8-9.0	1.8-12	3.4-11
<b>17α-OH Deficiency</b>														
<u>Pregnenolone</u>														
17-OH pregnenolone	0.3-0.7	0.3-5.0	0.2-0.7	0.2-1.3	0.17-0.7	0.03-0.3	0.1-2.9	0.1-0.5	0.3-3.6	0.2-1.5	0.2-2.8	0.2-0.9	0.1-1.7	0.1-1.0
<u>Progesterone</u>														
17-OH progesterone	0.2-1.8	0.1-1.5	–	–	0.2-2.1	0.4-1.1	0.2-5.2	0.4-1.1	0.2-3.5	0.5-1.5	0.2-2.6	0.2-0.9	0.2-2.2	0.2-1.5
<b>3β-HSD Deficiency</b>														
<u>17-OH pregnenolone</u>														
17-OH progesterone	1.1-5.2	3.6-11	1.8-6.5	3.6-12	2-22	3-20	2-22	2-20	0.3-3.0	0.5-3.3	0.5-6.0	0.3-5.3	0.4-3.4	0.5-6.3
<u>DHEA</u>														
Androstenedione	1.0-8.4	3.4-15	1.6-5.0	2.2-7.8	2.2-6.5	2.8-13	0.8-6.5	1.5-13	0.6-9.0	0.7-7.5	2.0-4.5	1.1-5.8	1.5-4.0	1.8-4.9
<b>Aldosterone Synthase Deficiency</b>														
<u>18-OH corticosterone</u>														
Aldosterone	1.0-4.5	0.8-2.6	1.1-10	1.2-11	1.3-5.0	2-13	1.3-5.0	2-13	1.2-6.0	1.9-15	2.6-7.1	5-12	2.0-5.7	3.4-13

21-OH, 21-hydroxylase; 11β-OH, 11β-hydroxylase; 17α-OH, 17α-hydroxylase; 3β-HSD, 3β-hydroxysteroid dehydrogenase.

\*Values in ng/dL except 11-deoxycortisol = ng/dL  
 ng/dL cortisol μg/dL.

Measurements by extraction, chromatography, and radioimmunoassay; data from references 20 and 22, and Quest Diagnostics Nichols Institute Clinical Correlations. Premature data derived during first week; measurements by extraction, chromatography, and radioimmunoassay; data from references 22 and 24.

Table 6. CAH Panel 11 Diagnostic Ratio Reference Ranges in Normal Neonates and Patients with CAH

Steroid Ratio	Age (Days)	Normals n = 59	21-OH Deficiency n = 32	3 $\beta$ -HSD Deficiency n = 2*	11 $\beta$ -OH Deficiency n = 2†	17 $\alpha$ -OH Deficiency n = 1‡
<b>21-OH Deficiency</b>						
<u>15<math>\beta</math>,17<math>\alpha</math>-(OH)2-pregnanolone x 100</u>	1	3.8-17.4	47.4-400			
Denominator	2-4	1.0-14.4	116-498			
	>16	1.0-2.6	170-1627	67.1; 150	33.6; 21.4	4.4
<u>17<math>\alpha</math>-OH-pregnanolone x 100</u>	1	2.0-22.0	29.9-423			
Denominator	2-4	1.0-11.0	103-974			
	>16	1.0-4.0	248-1470	74.8; 224	36.4; 22.0	8.0
<u>Pregnanetriol x 100</u>	1	2.0-30.0	36.6-648			
Denominator	2-4	1.0-13.0	112-286			
	>16	≤3.0	76.6-1344	22.6; 103	16.4; 5.8	4.1
<u>Pregnanetriolone x 100</u>	1	0.7-15.9	40.6-461			
Denominator	2-4	≤14.5	45.1-331			
	>16	≤0.2	83.3-961	8.3; 4.6	6.4; 3.4	12.9
<b>17<math>\alpha</math>-OH Deficiency</b>						
<u>5<math>\alpha</math>-THA x 100</u>	1	≤8.0	≤55.4			
Denominator	2-4	≤11.0	≤7.8			
	>16	≤34.3	≤75.4	ND; 57.0	ND; ND	50.4
<u>6<math>\alpha</math>-OH-THA x 100</u>	1	1.0-28.0	ND			
Denominator	2-4	1.0-11.0	ND			
	>16	1.0-2.0	ND	ND; ND	NMC; NMC	29983
<u>16<math>\alpha</math>-OH-pregnenolone</u>	1	0.3-1.3	1.2-4.4			
<u>16<math>\alpha</math>-Hydroxy-DHEA</u>	2-4	0.1-2.2	1.9-11.4			
	>16	0.2-0.7	1.0-11.7	0.9; 0.4	9.5; 8.1	156
<b>11<math>\beta</math>-OH Deficiency</b>						
<u>THS x 100</u>	1	2.9-25.0	13.6-34.0			
Denominator	2-4	1.1-26.0	10.4-49.1			
	>16	0.5-1.4	1.5-136	15.6; 27.0	366; 97.2	14.2
<u>6<math>\alpha</math>-OH-THS x 100</u>	1	≤13.0	ND			
Denominator	2-4	≤11.0	ND			
	>16	ND	ND	ND; ND	190,482; 117,723	ND
<b>3<math>\beta</math>-HSD Deficiency</b>						
<u>Pregnenetriol x 100</u>	1	≤24.4	≤27.4			
Denominator	2-4	≤17.1	≤36.6			
	>16	0.5-2.4	≤287.9	356; 149	36.4; 1.2	ND
<u>Pregnenetriol</u>	1	0.1-10.8	≤0.4			
<u>Pregnanetriolone</u>	2-4	0.2-95.2	≤0.4			
	>16	5.6-28.6	≤1.5	42.8; 32.5	2.6; 1.7	ND

21-OH, 21-hydroxylase; 17 $\alpha$ -OH, 17 $\alpha$ -hydroxylase; 11 $\beta$ -OH, 11 $\beta$ -hydroxylase; 3 $\beta$ -HSD, 3 $\beta$ -hydroxysteroid dehydrogenase; Denominator = sum of tetrahydrocortisone, alpha, and beta cortolone; THA = tetrahydro-compound A = tetrahydro-11-dehydrocorticosterone; ND = not detected, numerator level below detectable limit of assay; NMC = not measured due to contamination of numerator component; THS = tetrahydro-substance S = tetrahydro-11-deoxycortisol.

\*One patient; samples collected when 7 and 15 days of age.

†Two patients, 14 and 49 days of age.

‡One 7-day old patient.

Table 7. CAH Panel 11 Steroid Metabolite Reference Ranges

Steroid Metabolite	Lowest Limit of Quantitation (µg/g Creat)	1 Day Old (µg/g Creat)	2-4 Days Old (µg/g Creat)	>16 Days Old (µg/g Creat)
17α-Hydroxypregnanolone	10	30-420	15-200	40-90
15β,17α-Dihydroxypregnanolone	20	85-490	25-270	45-80
16α-Hydroxy-dehydroepiandrosterone (DHEA)	100	5000-220,000	2000-180,000	200-16,650
Pregnanetriol	2	45-620	20-280	5-70
Tetrahydro-11-deoxycortisol	10	70-670	30-470	15-60
15β,17α-Dihydroxypregnenolone	100	4900-43,900	1800-59,000	630-3300
Pregnanetriolone	2	20-280	≤260	≤10
16α-Hydroxypregnenolone	50	4400-98,900	3460-150,840	75-10,910
Pregnenetriol	10	≤360	≤390	20-180
Tetrahydrocortisone	100	620-13,160	530-7430	1600-5570
6α-Hydroxytetrahydro-11-deoxycortisol	40	≤120	≤170	<40
Tetrahydro-11-dehydrocorticosterone	150	*	*	320-1490
α-Cortolone	10	25-520	20-380	40-600
β-Cortolone	50	120-1360	150-1175	170-1350
6α-Hydroxytetrahydro-11-dehydrocorticosterone	10	30-540	20-250	10-100

\*Prior to 16 days of age, measurement of this steroid is compromised by interference from other fetal steroids.

## References

- Orth DN, Kovacs WJ. The adrenal cortex. In: Wilson, Foster, Kronenberg, et al. eds. *Williams Textbook of Endocrinology*. 9<sup>th</sup> ed. Philadelphia, PA: W.B. Saunders Company; 1998:598-607.
- Wajnrajch MP, New MI. Defects of adrenal steroidogenesis. In: DeGroot LJ, Jameson JL, eds. *Endocrinology*. 4<sup>th</sup> ed. Philadelphia, PA: W.B. Saunders Company; 2001:1721-1739.
- Speiser PW, White PC. Congenital adrenal hyperplasia. *N Engl J Med*. 2003;349:776-788.
- Speiser PW. Prenatal treatment of congenital adrenal hyperplasia. *J Urol*. 1999;162:5534-5536.
- White PC, Speiser PW. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Endocr Rev*. 2000;21:245-291.
- White PC, Curnow KM, Pascoe L. Disorders of steroid 11β-hydroxylase isozymes. *Endocr Rev*. 1994;15:421-438.
- Santos M, Kater CE, Auchus RJ, et al. Two prevalent *CYP17* mutations and genotype-phenotype correlations in 24 Brazilian patients with 17-hydroxylase deficiency. *J Clin Endocrinol Metab*. 2004;89:49-60.
- Azziz R, Dewailly D, Owerbach D. Clinical review 56: nonclassic adrenal hyperplasia: current concepts. *J Clin Endocrinol Metab*. 1994;78:810-815.
- Peter M, Partsch CJ, Sippell WG. Multisteroid analysis in children with terminal aldosterone biosynthesis defects. *J Clin Endocrinol Metab*. 1995;80:1622-1627.
- Chen X, Baker BY, Abduljabbar MA, et al. A genetic isolate of congenital lipid adrenal hyperplasia with atypical clinical findings. *J Clin Endocrinol Metab*. 2005;90:835-840.
- Arlt W, Walker EA, Draper N, et al. Congenital adrenal hyperplasia caused by mutant P450 oxidoreductase and human androgen synthesis: analytical study. *Lancet*. 2004;363:2128-2135.
- Fukami M, Horikawa R, Nagai T, et al. Cytochrome P450 oxidoreductase gene mutations and Antley-Bixler syndrome with abnormal genitalia and/or impaired steroidogenesis: molecular and clinical studies in 10 patients. *J Clin Endocrinol Metab*. 2005;90:414-423.
- Caulfield MP, Lynn TL, Gottschalk ME, et al. The diagnosis of congenital adrenal hyperplasia in the newborn by gas chromatography/mass spectrometry analysis of random urine specimens. *J Clin Endocrinol Metab*. 2002;87:3682-3690.
- Lutfallah C, Wang W, Mason JI, et al. Newly proposed hormonal criteria via genotypic proof for type II 3β-hydroxysteroid dehydrogenase deficiency. *J Clin Endocrinol Metab*. 2002;87:2611-2622.
- Mermejo LM, Elias LL, Marui S, et al. Refining hormonal diagnosis of type II 3β-hydroxysteroid dehydrogenase deficiency in patients with premature pubarche and hirsutism based on *HSD3B2* genotyping. *J Clin Endocrinol Metab*. 2005;90:1287-1293.

16. Azziz R, Zacur HA. 21-Hydroxylase deficiency in female hyperandrogenism: screening and diagnosis. *J Clin Endocrinol Metab.* 1989;69:577-584.
17. Siegel SF, Finegold DN, Lanes R, et al. ACTH stimulation tests and plasma dehydroepiandrosterone sulfate levels in women with hirsutism. *N Engl J Med.* 1990;323:849-854.
18. Azziz R, Bradley E, Huth J, et al. Acute adrenocorticotropin-(1-24) (ACTH) adrenal stimulation in eunorrhic women: reproducibility and effect of ACTH dose, subject weight, and sampling time. *J Clin Endocrinol Metab.* 1990;70:1273-1279.
19. Bridges NA, Hindmarch PC, Pringle PJ, et al. Cortisol, androstenedione (A4), dehydroepiandrosterone sulphate (DHEAS) and 17-hydroxyprogesterone (17OHP) responses to low doses of (1-24) ACTH. *J Clin Endocrinol Metab.* 1998;83:3750-3753.
20. Lee MM, Rajagopalan L, Berg GJ, et al. Serum adrenal steroid concentrations in premature infants. *J Clin Endocrinol Metab.* 1989;69:1133-1136.
21. Lashansky G, Saenger P, Fishman K, et al. Normative data for adrenal steroidogenesis in a healthy pediatric population: age- and sex-related changes after adrenocorticotropin stimulation. *J Clin Endocrinol Metab.* 1991;73:674-686.
22. Hingre RV, Gross SJ, Hingre KS, et al. Adrenal steroidogenesis in very low birth weight preterm infants. *J Clin Endocrinol Metab.* 1994;78:266-270.
23. Lashansky G, Saenger P, Dimartino-Nardi J, et al. Normative data for the steroidogenic response of mineralocorticoids and their precursors to adrenocorticotropin in a healthy pediatric population. *J Clin Endocrinol Metab.* 1992;75:1491-1496.
24. Adrenal steroid response to ACTH. Pediatrics, Endocrine Sciences, Calabasses Hills, CA. May 1991.



33608 Ortega Highway  
San Juan Capistrano, CA 92690-6130  
949-728-4000 800-642-4657

Quest, Quest Diagnostics, the associated logo, Nichols Institute, and all associated Quest Diagnostics marks are the trademarks of Quest Diagnostics.

© 2005 Quest Diagnostics Incorporated.  
All rights reserved.

[www.questdiagnostics.com](http://www.questdiagnostics.com)

CF1703-HS

6/2005