

## Overview

### Useful For

As an ancillary test for congenital adrenal hyperplasia (CAH), particularly in situations in which a diagnosis of 21-hydroxylase and 11-hydroxylase deficiency have been ruled out

Confirming a diagnosis of 3-beta-hydroxy dehydrogenase (3-beta-HSD) deficiency

Analysis for 17-hydroxypregnenolone is also useful as part of a battery of tests to evaluate females with hirsutism or infertility; both can result from adult-onset CAH.

### Testing Algorithm

See [Steroid Pathways](#) in Special Instructions.

### Special Instructions

- [Steroid Pathways](#)

### Method Name

Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

### NY State Available

Yes

## Specimen

### Specimen Type

Serum

### Specimen Required

**Collection Container/Tube:**

**Preferred:** Red top

**Acceptable:** Serum gel

**Submission Container/Tube:** Plastic vial

**Specimen Volume:** 1 mL

### Reject Due To

Gross hemolysis OK

Gross lipemia OK

Gross icterus OK

### Specimen Minimum Volume

0.5 mL

### Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Serum	Frozen (preferred)	28 days	

	Refrigerated	28 days	
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## Clinical & Interpretive

### Clinical Information

Congenital adrenal hyperplasia (CAH) is caused by inherited defects in steroid biosynthesis. Deficiencies in several enzymes cause CAH including 21-hydroxylase (*CYP21A2* mutations; 90% of cases), 11-hydroxylase (*CYP11A1* mutations; 5%-8%), 3-beta-hydroxy dehydrogenase (*HSD3B2* mutations; <5%), and 17-alpha-hydroxylase (*CYP17A1* mutations; 125 cases reported to date). The resulting hormone imbalances (reduced glucocorticoids and mineralocorticoids, and elevated steroid intermediates and androgens) can lead to life-threatening, salt-wasting crises in the newborn period and incorrect gender assignment of virilized females.

The adrenal glands, ovaries, testes, and placenta produce steroid intermediates, which are hydroxylated at the position 21 (by 21-hydroxylase) and position 11 (by 11-hydroxylase) to produce cortisol. Deficiency of either 21-hydroxylase or 11-hydroxylase results in decreased cortisol synthesis and loss of feedback inhibition of adrenocorticotrophic hormone (ACTH) secretion. The consequent increased pituitary release of ACTH drives increased production of steroid intermediates.

The steroid intermediates are oxidized at position 3 (by 3-beta-hydroxy dehydrogenase: 3-beta-HSD). The 3-beta-HSD enzyme allows formation of 17-hydroxyprogesterone (17-OHPG) from 17-hydroxypregnenolone and progesterone from pregnenolone. When 3-beta-HSD is deficient, cortisol is decreased, 17-hydroxypregnenolone and pregnenolone levels may increase, and 17-OHPG and progesterone levels, respectively, are low. Dehydroepiandrosterone (DHEA) is also converted to androstenedione by 3-beta-HSD and may be elevated in patients affected with 3-beta-HSD deficiency. The best screening test for CAH, most often caused by either 21- or 11-hydroxylase deficiency, is the analysis of 17-hydroxyprogesterone (along with cortisol and androstenedione). CAH21 / Congenital Adrenal Hyperplasia (CAH) Profile for 21-Hydroxylase Deficiency allows the simultaneous determination of these 3 analytes. Alternatively, these tests may be ordered individually: OHPG / 17-Hydroxyprogesterone, Serum; CIMP / Cortisol, Serum, LC-MS/MS; and ANST / Androstenedione, Serum.

If both 21- and 11-hydroxylase deficiency have been ruled out, analysis of 17-hydroxypregnenolone and pregnenolone may be used to confirm the diagnosis of 3-beta-HSD or 17-alpha-hydroxylase deficiency.

See [Steroid Pathways](#) in Special Instructions.

### Reference Values

#### CHILDREN\*

##### Males

Premature (26-28 weeks): 1,219-9,799 ng/dL

Premature (29-36 weeks): 346-8,911 ng/dL

Full term (1-5 months): 229-3,104 ng/dL

6 months-364 days: 221-1,981 ng/dL

1-2 years: 35-712 ng/dL

3-6 years: <277 ng/dL

7-9 years: <188 ng/dL

10-12 years: <393 ng/dL

13-15 years: 35-465 ng/dL

16-17 years: 32-478 ng/dL

#### TANNER STAGES

Stage I: <209 ng/dL

Stage II: <356 ng/dL

Stage III: <451 ng/dL

Stage IV-V: 35-478 ng/dL

Females

Premature (26-28 weeks): 1,219-9,799 ng/dL

Premature (29-36 weeks): 346-8,911 ng/dL

Full term (1-5 months): 229-3,104 ng/dL

6 months-364 days: 221-1,981 ng/dL

1-2 years: 35-712 ng/dL

3-6 years: <277 ng/dL

7-9 years: <213 ng/dL

10-12 years: <399 ng/dL

13-15 years: <408 ng/dL

16-17 years: <424 ng/dL

TANNER STAGES

Stage I: <236 ng/dL

Stage II: <368 ng/dL

Stage III: <431 ng/dL

Stage IV-V: <413 ng/dL

ADULTS

Males

> or =18 years: 55-455 ng/dL

Females

> or =18 years: 31-455 ng/dL

\*Kushnir MM, Rockwood AL, Roberts WL, et al: Development and performance evaluation of a tandem mass spectrometry assay for 4 adrenal steroids. Clin Chem 2006;52(8):1559-1567

### Interpretation

Diagnosis and differential diagnosis of congenital adrenal hyperplasia (CAH) always requires the measurement of several steroids. Patients with CAH due to steroid 21-hydroxylase gene (*CYP21A2*) mutations usually have very high levels of androstenedione, often 5-fold to 10-fold elevations. 17-hydroxyprogesterone (17-OHPG) levels are usually even higher, while cortisol levels are low or undetectable. All 3 analytes should be tested.

For the *HSD3B2* mutation, cortisol, 17-OHPG and progesterone levels will be decreased; 17-hydroxypregnenolone and pregnenolone and dehydroepiandrosterone (DHEA) levels will be increased.

In the much less common *CYP11A1* mutation, androstenedione levels are elevated to a similar extent as in *CYP21A2* mutation, and cortisol is also low, but OHPG is only mildly, if at all, elevated.

In the also very rare 17-alpha-hydroxylase deficiency, androstenedione, all other androgen-precursors (17-alpha-hydroxypregnenolone, OHPG, dehydroepiandrosterone sulfate), androgens (testosterone, estrone, estradiol), and cortisol are low, while production of mineral corticoid and its precursors (in particular pregnenolone, 11-dexycorticosterone, corticosterone, and 18-hydroxycorticosterone) are increased.

See [Steroid Pathways](#) in Special Instructions.

### Cautions

At birth, the hypothalamic-pituitary-adrenal axis and the hypothalamic-pituitary gonadal axis are activated and adrenal and sex steroid levels are high. In preterm infants, the elevations can be even more pronounced due to illness and stress. As a result, preterm infants may occasionally have 17-hydroxypregnenolone levels of up to 9,799 ng/dL. Term

infants (0-28 days) will have levels <3,104 ng/dL. These then fall over the following 2 years to prepubertal levels of <277 ng/dL.

**Supportive Data**

To convert to nmol/L, multiply the value in ng/dL by 0.03159757.

**Clinical Reference**

1. Wudy SA, Hartmann M, Svoboda M: Determination of 17-hydroxypregnenolone in plasma by stable isotope dilution/benchtop liquid chromatography-tandem mass spectrometry. *Horm Res* 2000;53(2):68-71
2. Therrell BL: Newborn screening for congenital adrenal hyperplasia *Endocrinol Metab Clin North Am* 2001;30(1):15-30
3. Bachega TA, Billerbeck AE, Marcondes JA, et al: Influence of different genotypes on 17-hydroxyprogesterone levels in patients with nonclassical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Clin Endocrinol* 2000;52(5):601-607
4. Kao P, Machacek DA, Magera MJ, et al: Diagnosis of adrenal cortical dysfunction by liquid chromatography-tandem mass spectrometry. *Ann Clin Lab Sci* 2001;31(2):199-204
5. Sciarra F, Tosti-Croce C, Toscano V: Androgen-secreting adrenal tumors. *Minerva Endocrinol* 1995;20(1):63-68
6. Collett-Solberg PF: Congenital adrenal hyperplasia: from genetics and biochemistry to clinical practice, part I. *Clin Pediatr* 2001;40(1):1-16

**Performance****Method Description**

Deuterium-labeled internal standards (pregnenolone-d4 and 17-hydroxypregnenolone-d3) are added to 0.2 mL of sample. Pregnenolone, 17-hydroxypregnenolone, and the internal standards are extracted from the sample using solid phase extraction. The extracts are then washed and dried under nitrogen. Extracts are then derivatized using hydroxylamine and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The mass spectrometer has an electrospray interface and is operated in the multiple-reaction monitoring positive mode. A 7-point standard curve is extracted and derivatized with each batch of samples. (Unpublished Mayo method)

**PDF Report**

No

**Specimen Retention Time**

14 days

**Performing Laboratory Location**

Rochester

**Fees & Codes****Test Classification**

This test was developed, and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the US Food and Drug Administration.

**CPT Code Information**

84143