MAYO CLINIC LABORATORIES

Test Definition: PREGN

Pregnenolone, S

Overview

Useful For

An ancillary test for congenital adrenal hyperplasia, 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 deficiency

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	

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 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 adrenocorticotropic 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 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-Hydoxylase Deficiency allows the simultaneous determination of these 3 analytes. Alternately, these tests may be ordered individually: OHPG / 17-Hydroxyprogesterone, Serum; CINP / 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

0-6 years: not established

7-9 years: <206 ng/dL

10-12 years: <152 ng/dL

13-15 years: 18-197 ng/dL

16-17 years: 17-228 ng/dL

Tanner Stages

Stage I: <157 ng/dL

Stage II: <144 ng/dL

Stage III: <215 ng/dL

Stage IV-V: 19-201 ng/dL

Females

0-6 years: not established

7-9 years: <151 ng/dL

10-12 years: 19-220 ng/dL

13-15 years: 22-210 ng/dL
16-17 years: 22-229 ng/dL
Tanner Stages
Stage I: <172 ng/dL
Stage II: 22-229 ng/dL
Stage III: 34-215 ng/dL
Stage IV-V: 26-235 ng/dL
ADULTS
> or =18 years: 33-248 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 require 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 will be decreased; 17-hydroxypregnenolone and pregnenolone and dehydroepiandrosterone levels will be increased.
In the much less common *CYP11A1* mutation, androstenedione levels are elevated to a similar extent as seen in *CYP21A2* mutation, and cortisol also is low, but 17-OHPG is only mildly, if at all, elevated.
In the also very rare 17-hydroxylase deficiency, androstenedione, all other androgen-precursors (17-alpha-hydroxypregnenolone, 17-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

No significant cautionary statements

Supportive Data

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

Clinical Reference

1. Wudy S A, Hartmann M, Svoboda M: Determination of 17-hydroxyprogesterone 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 (Oxf) 2000:52(5):601-607
4. Kao P, Machacek DA, Magera MJ at 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 1. Clin Pediatr (Phila) 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

84140