MAYO CLINIC LABORATORIES

# Test Definition: PREGN

Pregnenolone, Serum

## Overview

### Useful For

An ancillary test for congenital adrenal hyperplasia, particularly in situations in which a diagnosis of both 21- and 11-hydroxylase deficiency have been ruled out

Confirming a diagnosis of 3-beta-hydroxy dehydrogenase deficiency

### Testing Algorithm

For more information see Steroid Pathways.

### Special Instructions

- Steroid Pathways

### Method Name

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

### NY State Available

Yes

## Specimen

### Specimen Type

Serum

### Specimen Required

**Supplies:** Sarstedt Aliquot Tube, 5 mL (T914)
**Collection Container/Tube:**
**Preferred:** Red top
**Acceptable:** Serum gel
**Submission Container/Tube:** Plastic vial
**Specimen Volume:** 1 mL
**Collection Instructions:**
1. Within 2 hours of collection, centrifuge the specimen and immediately aliquot serum into a plastic vial.
2. Freeze immediately.

### Specimen Minimum Volume

0.5 mL

### Reject Due To

| | |
|---|---|
| Gross hemolysis | OK |
| Gross lipemia | OK |
| Gross icterus | OK |

### Specimen Stability Information

| Specimen Type | Temperature | Time | Special Container |
|---|---|---|---|
| Serum | Frozen | 28 days | |

## Clinical & Interpretive

### Clinical Information

Congenital adrenal hyperplasia (CAH) is caused by inherited defects in steroid biosynthesis. Deficiencies in several enzymes can cause CAH, including 21-hydroxylase (*CYP21A2* variants; 90% of cases), 11-hydroxylase (*CYP11A1* variants; 5%-8%), 3-beta-hydroxysteroid dehydrogenase (3-beta-HSD) (*HSD3B2* variants; <5%), and 17-alpha-hydroxylase (*CYP17A1* variants; 125 cases reported to date). The resulting hormone imbalances (reduced glucocorticoids and mineralocorticoids; 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 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-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 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-OHPG, along with cortisol and androstenedione. CAH21 / Congenital Adrenal Hyperplasia (CAH) Profile for 21-Hydroxylase Deficiency, Serum allows the simultaneous determination of these 3 analytes. Alternatively, these tests may be ordered individually: OHPG / 17-Hydroxyprogesterone, Serum; CINP / Cortisol, Mass Spectrometry, Serum; 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.

For more information see Steroid Pathways.

### Reference Values

CHILDREN/ADOLESCENTS*
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

*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

ADULTS
> or =18 years: 33-248 ng/dL

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

## Interpretation

The 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*) variants 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* variant, cortisol, 17-OHPG, and progesterone levels will be decreased; 17-hydroxypregnenolone, pregnenolone, and dehydroepiandrosterone (DHEA) levels will be increased.

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

In the 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 (particularly pregnenolone, 11-dexycorticosterone, corticosterone, and 18-hydroxycorticosterone) are increased.

For more information see Steroid Pathways.

### Cautions

No significant cautionary statements

### 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. Kushnir MA, Rockwood AL, Roberts WL, et al: Development and performance evaluation of a tandem mass spectrometry assay for 4 adrenal steroids. Clinical Chemistry 2006;52(8):1559-1567
6. Collett-Solberg PF. Congenital adrenal hyperplasia: from genetics and biochemistry to clinical practice, Part 1. Clin Pediatr (Phila). 2001:40(1):1-16
7. Chormanski D, Muzio MR. C 17 hydroxylase deficiency. In: StatPearls [Internet]. StatPearls Publishing; 2021. Updated January 3, 2023. Accessed May 29, 2024. Available at www.ncbi.nlm.nih.gov/books/NBK546644/

## 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. The mass spectrometer has an electrospray interface and is operated in the multiple-reaction monitoring positive mode. A 6-point standard curve is extracted and derivatized with each batch of samples.(Unpublished Mayo method)

### PDF Report

No

### Day(s) Performed

Monday through Wednesday, Friday

### Report Available

3 to 7 days

### Specimen Retention Time
14 days

### Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Superior Drive

## Fees & Codes

### Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

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

### CPT Code Information
84140

### LOINC® Information

| Test ID | Test Order Name | Order LOINC® Value |
|---|---|---|
| PREGN | Pregnenolone, S | 2837-3 |

| Result ID | Test Result Name | Result LOINC® Value |
|---|---|---|
| 88645 | Pregnenolone, S | 2837-3 |