

## Overview

### Useful For

Second-tier testing of newborns with abnormal screening result for congenital adrenal hyperplasia

### Genetics Test Information

This test is a second-tier newborn screen for the diagnosis of congenital adrenal hyperplasia.

### Special Instructions

- [Blood Spot Collection Card-Spanish Instructions](#)
- [Blood Spot Collection Card-Chinese Instructions](#)
- [Blood Spot Collection Instructions](#)

### Method Name

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

Portions of this test are covered by patents held by Quest Diagnostics

### NY State Available

Yes

## Specimen

### Specimen Type

Whole blood

### Necessary Information

**Birth weight, time of birth, and gestational age are required.**

### Specimen Required

**Supplies:** Card-Blood Spot Collection (Filter Paper) (T493)

**Container/Tube:**

**Preferred:** Blood Spot Collection Card

**Acceptable:** Local newborn screening card, Whatman 903 filter paper, PerkinElmer 226 filter paper, Munktell filter paper

**Specimen Volume:** 2 Blood spots

**Collection Instructions:**

1. Do not use device or capillary tube containing EDTA to collect specimen.
2. Completely fill at least 2 circles on the filter paper card (approximately 100 microliters blood per circle).
3. Let blood dry on filter paper at ambient temperature in a horizontal position for a minimum of 3 hours.
4. Do not expose specimen to heat or direct sunlight.
5. Do not stack wet specimens.

6. Keep specimen dry.

**Additional Information:**

1. For collection instructions, see [Blood Spot Collection Instructions](#)
2. For collection instructions in Spanish, see [Blood Spot Collection Card-Spanish Instructions](#) (T777)
3. For collection instructions in Chinese, see [Blood Spot Collection Card-Chinese Instructions](#) (T800)

**Forms**

[If not ordering electronically, complete, print, and send a Biochemical Genetics Test Request](#) (T798) with the specimen.

**Specimen Minimum Volume**

1 Blood spot

**Reject Due To**

Blood spot specimen that shows serum rings or has multiple layers	Reject
Insufficient specimen	Reject
Unapproved filter papers	Reject

**Specimen Stability Information**

Specimen Type	Temperature	Time	Special Container
Whole blood	Ambient (preferred)	90 days	FILTER PAPER
	Refrigerated	90 days	FILTER PAPER
	Frozen	90 days	FILTER PAPER

**Clinical & Interpretive****Clinical Information**

Congenital adrenal hyperplasia (CAH) is a group of disorders caused by inherited defects in steroid biosynthesis, most commonly, 21-hydroxylase deficiency (approximately 90% of cases) and 11-beta hydroxylase deficiency (approximately 5% of cases). The overall incidence of CAH due to 21-hydroxylase deficiency is approximately 1 in 15,000 live births. Individuals with CAH may present with life-threatening salt-wasting crises in the newborn period and incorrect sex assignment of virilized females, which occurs due to in utero exposure to reduced glucocorticoids and mineralocorticoids and elevated 17-hydroxyprogesterone (17-OHP) and androgens. Hormone replacement therapy, when initiated early, results in a significant reduction in morbidity and mortality. Therefore, newborn screening for CAH is desirable and has been implemented in all 50 states.

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Immunoassays are typically used to quantify 17-OHP as a marker for CAH in the newborn screen setting. However, these immunoassays are hampered by cross-reactivity of the antibodies with other steroids, yielding a high rate of false-positive results. Tandem mass spectrometry allows for the simultaneous specific determination of 17-OHP and other steroids, such as androstenedione, cortisol, 11-deoxycortisol, and 21-deoxycortisol. Application of this technology to the determination of steroids in newborn blood spots significantly enhances the correct identification of patients with CAH and reduces the number of false-positive screening results when implemented as a second-tier analysis performed prior to reporting of initial newborn screen results.

**Reference Values**

17-Hydroxyprogesterone (17-OHP)

<15.1 ng/mL

Androstenedione

<3.1 ng/mL

Cortisol

Not applicable

11-Deoxycortisol

<15.1 ng/mL

21-Deoxycortisol

<4.1 ng/mL

(17-OHP + Androstenedione)/Cortisol Ratio

<1.1

**Note:** Abnormal (17-OHP + Androstenedione)/Cortisol Ratio: > or =1.1 is only applicable when 17-OHP is elevated

11-Deoxycortisol/Cortisol Ratio

Not applicable

**Interpretation**

Findings of a 17-hydroxyprogesterone (17-OHP) value greater than 15.0 ng/mL and a high (17-OHP + androstenedione)/cortisol ratio (> or =1) are supportive of the initial abnormal newborn screening result.

Findings of an 11-deoxycortisol value greater than 15.0 ng/mL or 21-deoxycortisol greater than 4.0 ng/mL with elevated 17-OHP further support the abnormal newborn screening result and increase the diagnostic specificity. Clinical and laboratory follow-up is strongly recommended.

**Cautions**

This is a screening test, and while its positive predictive value is significantly higher than that of immunoassays (9.0% versus 0.5%), false-positive results can occur. Follow-up of abnormal results is necessary; order OHPG / 17-Hydroxyprogesterone, Serum and DOCS / 11- Deoxycorticosterone, Serum.

Deoxycorticosterone (DOC) coelutes with 17-hydroxyprogesterone (17-OHP). DOC is a precursor of aldosterone in the

steroidogenesis pathway. It is not elevated in patients with classic congenital adrenal hyperplasia (CAH) due to 21-alpha-hydroxylase deficiency; indeed, its production is lacking in this deficiency. However, DOC is expected to be elevated in other variants of CAH, specifically 11-alpha-hydroxylase deficiency (which also includes elevations of 11-deoxy) and 17-alpha-hydroxylase/17,20-lyase deficiency (none of the other targeted steroids are elevated in this condition). Therefore, if this assay was applied as a first-tier screening assay, a finding of elevated DOC and/or 17-OHP can uncover clinically relevant variants of CAH that would be confirmed through follow-up testing. Because this assay's goal is to identify patients with CAH but not exclusively 17-alpha-hydroxylase deficiency, the separation of 17-OHP and DOC during screening is not essential and may lead to a quicker diagnosis of at-risk patients for these rarer variants of CAH. However, as a second-tier test performed only when the first-tier test for 17-OHP is abnormal, 17-alpha-hydroxylase/17,20-lyase deficiency will not be uncovered because 17-OHP is not elevated in that condition.

**Clinical Reference**

1. Claahsen-van der Grinten HL, Speiser PW, et al. Congenital adrenal hyperplasia-Current insights in pathophysiology, diagnostics, and management. *Endocr Rev.* 2022;43(1):91-159. doi:10.1210/endrev/bnab016
2. Minutti CZ, Lacey JM, Magera MJ, et al. Steroid profiling by tandem mass spectrometry improves the positive predictive value of newborn screening for congenital adrenal hyperplasia. *J Clin Endo Met.* 2004;89(8):3687-3693
3. Turcu AF, Auchus RJ. The next 150 years of congenital adrenal hyperplasia. *J Steroid Biochem Mol Biol.* 2015;153:63-714
4. Witchel SF, Azziz R. Congenital adrenal hyperplasia. *Pediatr Adolesc Gynecol.* 2011;24(3):116-126
5. Nimkarn S, Gangishetti PK, Yau M, et al. 21-Hydroxylase-Deficient Congenital Adrenal Hyperplasia.. In: Adam MP, Feldman J, Mirzaa GM, et al, eds. *GeneReviews* [Internet].University of Washington, Seattle; 1993-2025. Updated February 4. 2016. Accessed July 11, 2025. Available at [www.ncbi.nlm.nih.gov/books/NBK1171/](http://www.ncbi.nlm.nih.gov/books/NBK1171/)

**Performance****Method Description**

A 1/8-inch disk is punched out of the blood spot into a 96-well filter plate. Internal standards are added to the filter plate. The punched disks are eluted and centrifuged, then dried under nitrogen and reconstituted with water:methanol. Analysis is by electrospray liquid chromatography-tandem mass spectrometry. The concentration of 17-hydroxyprogesterone, androstenedione, cortisol, 11-deoxycortisol, and 21-deoxycortisol are established by comparison of their ion intensity to that of their respective internal standards.(Unpublished Mayo method)

**PDF Report**

No

**Day(s) Performed**

Monday through Saturday

**Report Available**

1 to 3 days

**Specimen Retention Time**

2 years

**Performing Laboratory Location**

Mayo Clinic Laboratories - Rochester Main Campus

**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**

82542

**LOINC® Information**

Test ID	Test Order Name	Order LOINC® Value
CAH2T	CAH Newborn Screen, BS	57086-1

Result ID	Test Result Name	Result LOINC® Value
42207	17-OH Progesterone	38473-5
42208	Androstenedione	53343-0
42209	Cortisol	53345-5
42210	11-deoxycortisol	53338-0
42211	21-deoxycortisol	53341-4
42212	(17OHP+Androstenedione)/Cortisol	53336-4
42213	11-deoxycortisol/Cortisol	No LOINC Needed
42214	Interpretation (CAH2T)	46758-9
BG688	Birth Weight (grams, XXXX)	8339-4
BG689	Time of Birth (24hr time, XX:XX)	57715-5
BG690	Gestational Age (weeks, XX.X)	76516-4
42206	Reviewed By	18771-6