Test Definition: CAH2T

Congenital Adrenal Hyperplasia Newborn Screen, Blood Spot

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.

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. Pediatri 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/

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

Test Definition: CAH2T

Congenital Adrenal Hyperplasia Newborn Screen, Blood Spot

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