Test Definition: CYPZ

21-Hydroxylase Gene, CYP21A2, Full Gene Analysis, Varies

Overview

Useful For

Carrier screening and diagnosis of 21-hydroxylase deficient congenital adrenal hyperplasia (CAH) in individuals with a personal or family history of 21-hydroxylase deficiency, or as follow-up to positive CAH newborn screens and/or measurement of basal and adrenocorticotropic hormone- 1-24 stimulated 17-hydroxyprogesterone, androstenedione, and other adrenal steroid levels

May identify *CYP21A2* variants in individuals with a suspected diagnosis of 21-hydroxylase deficient CAH when a common variant panel is negative or only identifies 1 variant

May identify the CH-1 *TNXA::TNXB* hybrid associated with CAH-X. Note that the CH-2 and CH-3 *TNXA::TNXB* hybrids will not be detected by current methodologies.

Evaluation of 21-hydroxlase deficiency in prenatal cases with suspected differences of sex development (such as clitoromegaly) detected by ultrasound, particularly when the fetus is confirmed XX female by chromosome analysis

Due to the complexity of the *CYP21A2* locus, site specific testing for known/familial *CYP21A2* variants is not offered.

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
MATCC	Maternal Cell Contamination, B	Yes	No
CULFB	Fibroblast Culture for Genetic Test	Yes	No
CULAF	Amniotic Fluid Culture/Genetic Test	Yes	No
_STR1	Comp Analysis using STR (Bill only)	No, (Bill only)	No
_STR2	Add'l comp analysis w/STR (Bill Only)	No, (Bill only)	No

Genetics Test Information

This test includes Sanger sequencing, multiplex ligation-dependent probe amplification, and a droplet digital polymerase chain reaction assay (as needed) to evaluate the *CYP21A2* gene for carrier screening and diagnosis of 21-hydroxylase deficient congenital adrenal hyperplasia (CAH).

Testing Algorithm

For prenatal specimens only:
If amniotic fluid (nonconfluent cultured cells) is received, amniotic fluid culture will be added and performed at an additional charge.

If chorionic villus specimen (nonconfluent cultured cells) is received, fibroblast culture will be added and performed at an additional charge.

For any prenatal specimen that is received, maternal cell contamination studies will be added and performed at an additional charge.

Special Instructions

- Informed Consent for Genetic Testing
- CYP21A2 Gene Testing for Congenital Adrenal Hyperplasia Patient Information
- Informed Consent for Genetic Testing (Spanish)
- CYP21A2 Gene Analysis: Frequently Asked Questions

Highlights

This test aids in carrier screening and diagnosis of 21-hydroxylase deficient congenital adrenal hyperplasia (CAH).

Full gene sequencing, multiplex ligation-dependent probe amplification, and droplet digital polymerase chain reaction (when needed) are used to detect the common disease-causing *CYP21A2* variants, *CYP21A2* full gene deletions, and rare *CYP21A2* variants.

Method Name

Polymerase Chain Reaction (PCR) Amplification followed by DNA Sequence Analysis and Gene Dosage Analysis by Multiplex Ligation-Dependent Probe Amplification (MLPA) and Droplet Digital PCR (ddPCR)

NY State Available

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

This test is a molecular analysis of the *CYP21A2* gene and does not include biochemical analysis of steroids. For biochemical analysis for congenital adrenal hyperplasia (CAH), which includes cortisol, androstenedione, and 17-hydroxyprogesterone, see CAH21 / Congenital Adrenal Hyperplasia (CAH) Profile for 21-Hydroxylase Deficiency, Serum.

For answers to frequently asked questions, see CYP21A2 Gene Analysis: Frequently Asked Questions.

Additional Testing Requirements

All prenatal specimens must be accompanied by a maternal blood specimen; order MATCC / Maternal Cell

Contamination, Molecular Analysis, Varies on the maternal specimen.

Necessary Information

CYP21A2 Gene Testing for Congenital Adrenal Hyperplasia Patient Information (T663) is strongly recommended, but not required, to be filled out and sent with the specimen. This information aids in providing a more thorough interpretation of test results. Ordering healthcare professionals are strongly encouraged to complete the form and send it with the specimen.

Specimen Required

Patient Preparation: A previous bone marrow transplant from an allogenic donor will interfere with testing. For information about testing patients who have received a bone marrow transplant, call 800-533-1710.

Submit only 1 of the following specimens:

Specimen Type: Whole blood
Container/Tube:
Preferred: Lavender top (EDTA) or yellow top (ACD)
Acceptable: None
Specimen Volume: 3 mL
Collection Instructions:
1. Invert several times to mix blood.
2. Send whole blood specimen in original tube. **Do not aliquot.**
3. Whole blood collected postnatal from an umbilical cord is also acceptable. See Additional Information
Specimen Stability Information: Ambient 4 days (preferred)/Refrigerated 4 days/Frozen 4 days
Additional Information:
1. Specimens are preferred to be received within 4 days of collection. Extraction will be attempted for specimens received after 4 days, and DNA yield will be evaluated to determine if testing may proceed.
2. To ensure minimum volume and concentration of DNA are met, the requested volume must be submitted. Testing may be canceled if DNA requirements are inadequate.
3. For postnatal umbilical cord whole blood specimens, maternal cell contamination studies are recommended to ensure test results reflect that of the patient tested. A maternal blood specimen is required to complete maternal cell contamination studies. Order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on both the cord blood and maternal blood specimens under separate order numbers.

Specimen Type: Extracted DNA
Container/Tube:
Preferred: Screw Cap Micro Tube, 2 mL with skirted conical base
Acceptable: Matrix tube, 1 mL
Collection Instructions:
1. The preferred volume is at least 100 mcL at a concentration of 75 ng/mcL.
2. Include concentration and volume on tube.
Specimen Stability Information: Frozen (preferred) 1 year/Ambient/Refrigerated
Additional Information: DNA must be extracted in a CLIA-certified laboratory or equivalent and must be extracted from a specimen type listed as acceptable for this test (including applicable anticoagulants). Our laboratory has experience

with Chemagic, Puregene, Autopure, MagnaPure, and EZ1 extraction platforms and cannot guarantee that all extraction methods are compatible with this test. If testing fails, one repeat will be attempted, and if unsuccessful, the test will be reported as failed and a charge will be applied. If applicable, specific gene regions that were unable to be interrogated due to DNA quality will be noted in the report.

Prenatal Specimens
Due to its complexity, consultation with the laboratory is required for all prenatal testing; call 800-533-1710 to speak to a genetic counselor.

Preferred:
Specimen Type: Amniotic fluid
Container/Tube: Amniotic fluid container
Specimen Volume: 20 mL
Specimen Stability Information: Refrigerated (preferred) <24 hours/Ambient <24 hours
Additional information:
1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
2. A separate culture charge will be assessed under CULAF / Culture for Genetic Testing, Amniotic Fluid. An additional 2 to 3 weeks is required to culture amniotic fluid before genetic testing can occur.
3. **All prenatal specimens must be accompanied by a maternal blood specimen**; order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen.

Specimen Type: Chorionic villi
Container/Tube: 15-mL tube containing 15 mL of transport media
Specimen Volume: 20 mg
Specimen Stability Information: Refrigerated (preferred) <24 hours/Ambient <24 hours
Additional Information:
1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing. An additional 3 to 4 weeks is required to culture fibroblasts before genetic testing can occur.
3. **All prenatal specimens must be accompanied by a maternal blood specimen;** order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen.

Specimen Type: Prenatal cultured fibroblasts (eg, products of conception), amniocytes, or other confluent cultured cells. This does not include cultured chorionic villi.
Container/Tube: T-25 flask
Specimen Volume: 2 Flasks
Collection Instructions: Submit confluent cultured cells from another laboratory.
Specimen Stability Information: Ambient (preferred) <24 hours/Refrigerated <24 hours)
Additional Information:
1. Specimens are preferred to be received within 24 hours of collection. Culture and extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
2. A separate culture charge will be assessed under CULAF / Culture for Genetic Testing, Amniotic Fluid or CULFB /

Fibroblast Culture for Biochemical or Molecular Testing.
3. **All prenatal specimens must be accompanied by a maternal blood specimen;** order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen.

Specimen Type: Cultured chorionic villi
Container/Tube: T-25 flasks
Specimen Volume: 2 Full flasks
Collection Instructions: Submit confluent cultured cells from another laboratory.
Specimen Stability Information: Ambient (preferred) <24 hours/Refrigerated <24 hours
Additional Information:
1. Specimens are preferred to be received within 24 hours of collection. Culture and/or extraction will be attempted for specimens received after 24 hours and will be evaluated to determine if testing may proceed.
2. A separate culture charge will be assessed under CULFB / Fibroblast Culture for Biochemical or Molecular Testing
3. **All prenatal specimens must be accompanied by a maternal blood specimen;** order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen.

Forms
1. **New York Clients-Informed consent is required.** Document on the request form or electronic order that a copy is on file. The following documents are available:
-Informed Consent for Genetic Testing (T576)
-Informed Consent for Genetic Testing-Spanish (T826)
2. *CYP21A2* Gene Testing for Congenital Adrenal Hyperplasia Patient Information (T663) is recommended.
3. If not ordering electronically, complete, print, and send a Biochemical Genetics Test Request (T798) with the specimen.

Specimen Minimum Volume
See Specimen Required.

Reject Due To
All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies		

Clinical & Interpretive

Clinical Information
Congenital adrenal hyperplasia (CAH), with a worldwide incidence rate of 1 in 14,000 to 1 in 18,000 live births, is one of the most common inherited conditions. It is characterized by impaired cortisol production due to inherited defects in steroid biosynthesis. The clinical consequences of CAH, besides diminished cortisol production, depend on which enzyme is affected and whether the loss of function is partial or complete.

In greater than 90% of CAH cases, the affected enzyme is 21-steroid hydroxylase, encoded by the *CYP21A2* gene located on chromosome 6. 21-hydroxylase deficient CAH (21-OHD CAH) is inherited in an autosomal recessive pattern and has a spectrum of clinical symptoms depending upon residual enzyme activity. Excessive adrenal androgen biosynthesis results in varying degrees of virilization. If there is approximately 20% to 50% residual enzyme activity a non-classic phenotype results, with signs of hyperandrogenism typically starting in later childhood or adolescence. Individuals with severe enzyme deficiency have the classic form of CAH, with prenatal onset of virilization. Classic CAH is further subdivided into simple-virilizing (generally between 1% to 5% residual enzyme activity) and salt-wasting (<1% residual enzyme activity) forms. Patients with salt-wasting CAH have both cortisol and mineral corticosteroid deficiency and are at risk for life-threatening salt-wasting crises if untreated.

Because of its high incidence rate, 21-hydroxylase deficiency is included in most US newborn screening programs, typically by measuring 17-hydroxyprogesterone concentrations in blood spots by immunoassay. Confirmation by other testing strategies (eg, liquid chromatography tandem mass spectrometry [LC-MS/MS], CAH2T / Congenital Adrenal Hyperplasia Newborn Screen, Blood Spot), or retesting after several weeks, is required for most positive screens because of the high false-positive rates of the immunoassays (due to physiological elevations of 17-hydroxyprogesterone in premature babies and immunoassay cross-reactivity with other steroids). In a small percentage of cases, additional testing will fail to provide a definitive diagnosis. In addition, screening strategies can miss non-classic CAH cases, which may present later in childhood or adolescence and require more extensive steroid hormone profiling, including testing before and after adrenal stimulation with corticotropin (previously adrenocorticotropic hormone: ACTH)-1-24. In some non-classic CAH cases individuals may not come to medical attention until adulthood. Rare instances of cases of classic CAH being missed by newborn screening have also been reported.

For these reasons, molecular genetic testing plays an important role in both classic and non-classic CAH cases. In addition, the high carrier frequency (approximately 1 in 50) for *CYP21A2* variants makes genetic diagnosis important for genetic counseling and risk assessment. Genetic testing can also play a role in prenatal diagnosis of 21-hydroxylase deficiency. However, accurate genetic diagnosis continues to be a challenge because most of the variants arise from recombination events between *CYP21A2* and its highly homologous pseudogene, *CYP21A1P* (transcriptionally inactive). In particular, unequal crossovers and gene conversion events result in large structural rearrangements, copy number changes, and sequence transfers between *CPY21A2* and *CYP21A1P*. Approximately, 90% of individuals with 21-OHD CAH have one or more common pseudogene derived pathogenic variants.

The high likelihood of an affected individual having a common variant means that some laboratories may offer genotyping assays, particularly in the setting of carrier screening, which may miss disease causing variants, or be unable to determine cis/trans status of detected variants. Full gene sequencing and copy number assessment of *CYP21A2* is the best approach to exclude or confirm a diagnosis of 21-OHD CAH. However, the high homology between *CYP21A2* and its pseudogene, the high probability of hybrid or chimera alleles, and frequent copy number gains and losses, present diagnostic challenges, including for most short-read next generation sequencing technologies. Therefore, comprehensive genetic testing strategies should consider all of the above challenges when assessing *CYP21A2* for disease causing variants. Testing of additional family members may be necessary to clarify the phase of identified reportable variants (i.e. whether the variants are in cis in the same copy of *CYP21A2* or in trans on different copies).

In addition, recent years have seen an uptick in literature focusing on CAH-X syndrome, which presents when an unequal crossover event results in a full gene *CYP21A2* deletion and a *TNXA::TNXB* hybrid. It is estimated that approximately 6% to15% of individuals with a diagnosis of 21-OHD CAH have CAH-X and these individuals are more likely to have

hypermobile Ehlers Danlos syndrome features in addition to classic CAH features. These additional symptoms may include joint hypermobility, chronic joint pain, joint dislocations, and cardiac valve abnormalities. There are 3 CAH-X *TNXA*::*TNXB* hybrids that have been reported in the literature: CH-1, CH-2 and CH-3; all of these hybrids are associated with a full gene deletion of *CYP21A2*.

Reference Values

An interpretive report will be provided.

Interpretation

All detected alterations will be evaluated according to American College of Medical Genetics and Genomics (ACMG) recommendations.(1) Variants will be classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance.

Cautions

Because of the complexity of the genetic structure of the *CYP21A2* locus, and the possibility that a patient's congenital adrenal hyperplasia (CAH) may be due to other gene defects, genetic testing results should be correlated carefully with clinical and biochemical data.

This testing strategy is superior to approaches previously used but may still miss some complex and large-scale genetic rearrangements or deletions, as well as genetic changes in far upstream or downstream gene-regulatory elements that impair *CYP21A2* gene expression. This can lead to false-negative test results.

Rare variants in primer binding sites can lead to selective allelic drop-out, which can lead to false-negative or false-positive results.

Patients without genetic evidence for disease-causing *CYP21A2* genetic changes may still have CAH due to a different enzyme defect. Additional and expanded biochemical steroid profiling is recommended if the clinical picture is strongly suggestive of CAH.

While results may be suggestive of a *TNXA::TNXB* CH-1 hybrid, CH-2 and CH-3 hybrids will be missed, and this test is not designed to definitively detect CAH-X.

Clinical Reference

1. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-424
2. Collett-Solberg PF. Congenital adrenal hyperplasias: from clinical genetics and biochemistry to clinical practice, part I. Clin Pediatr. 2001;40:1-16
3. Mercke DP, Bornstein SR, Avila NA, Chrousos GP. NIH conference: future directions in the study and management of congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Ann Intern Med. 2002;136:320-334
4. Speiser PW, White PC. Medical progress: congenital adrenal hyperplasia. N Engl J Med. 2003;349:776-788
5. Sarafoglou K, Banks K, Kyllo J, Pittock S, Thomas W. Cases of congenital adrenal hyperplasia missed by newborn screening in Minnesota. JAMA. 2012;307(22):2371-2374
6. Lao Q, Brookner B, Merke DP. High-Throughput Screening for CYP21A1P-TNXA/TNXB Chimeric Genes Responsible for Ehlers-Danlos Syndrome in Patients with Congenital Adrenal Hyperplasia. J Mol Diagn. 2019;21(5):924-931

7. Baumgartner-Parzer S, Witsch-Baumgartner M, Hoeppner W. EMQN best practice guidelines for molecular genetic testing and reporting of 21-hydroxylase deficiency. Eur J Hum Genet. 2020;28(10):1341-1367
8. Concolino P, Falhammar H. CAH-X Syndrome: Genetic and Clinical Profile. Mol Diagn Ther. 2022;26(3):293-300
9. Claahsen-van der Grinten HL, Speiser PW, Ahmed SF, et al. Congenital Adrenal Hyperplasia-Current Insights in Pathophysiology, Diagnostics, and Management. Endocr Rev. 2022;43(1):91-159

Performance

Method Description

A combined testing approach involving polymerase chain reaction (PCR) amplification, bi-directional sequence analysis, multiplex ligation-dependent probe amplification (MLPA), and droplet digital (ddPCR), when necessary, is used to identify sequence variants and copy number variation within the *CYP21A2* gene (GenBank accession number NM_000500; build GRCh37 [hg19]).

Four sets of PCR primer pairs amplify the *CYP21A2* gene, the inactive *CYP21A1P* pseudogene, and the *CYP21A2::CYP21A1P* and *CYP21A1P::CYP21A2* hybrids to determine the presence or absence of amplification product.

Bi-directional full gene sequence analysis, including a portion of the promoter, 5'-untranslated region, and 3'-untranslated region, is then performed on the *CYP21A2* gene and the *CYP21A2::CYP21A1P* hybrid (if present) to test for the presence of sequence variants. Because the *CYP21A1P::CYP21A2* hybrid and the *CYP21A1P* pseudogene are expected to be inactive, sequencing is not performed unless required for interpretation.

Multiplex ligation-dependent probe amplification is performed to determine the copy number of the 5-prime and 3-prime regions of the *CYP21A2* gene and the *CYP21A1P* pseudogene. In certain cases, a ddPCR assay is used in conjunction with MLPA to determine copy number. Quantification and comparison of results is used to determine the copy number of the *CYP21A2* gene, the *CYP21A1P* pseudogene, and the *CYP21A2::CYP21A1P* and *CYP21A1P::CYP21A2* hybrids.

Correlation of all performed test components is used to determine the *CYP21A2* genotype.
This technology cannot always determine the cis/trans status (cis:=same chromosome, trans:=opposite chromosomes) of the identified genes, rearrangements, or variants. Family studies of blood relatives may assist in determination of the cis/trans status.(Cradic KW, Grebe SK. A diagnostic sequencing assay for CYP21 based on promoter activity provides better understanding of gene rearrangements. Abstract. Endocrine Society Annual Meeting, ENDO 2005)

PDF Report

No

Day(s) Performed

Varies

Report Available

14 to 21 days

Specimen Retention Time

Whole blood: 30 days (if available); Extracted DNA: 90 days

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

81405-CYP21A2 (cytochrome P450, family 21, subfamily A, polypeptide2) (eg, steroid 21-hydroxylase isoform, congenital adrenal hyperplasia), full gene sequence
81402-CYP21A2 (cytochrome P450, family 21, subfamily A, polypeptide2) (eg, congenital adrenal hyperplasia, 21-hydroxylase deficiency), common variants (eg, IVS2-13G, P30L, I172N, exon 6 mutation cluster [I235N, V236E, M238K], V281L, L307FfsX6, Q318X, R356W, P453S, G110VfsX21, 30-kb deletion variant)
88233-Tissue culture, skin or solid tissue biopsy (if appropriate)
88235-Tissue culture for amniotic fluid (if appropriate)
88240-Cryopreservation (if appropriate)
81265-Comparative analysis using Short Tandem Repeat (STR) markers; patient and comparative specimen (eg, pre-transplant recipient and donor germline testing, post-transplant non-hematopoietic recipient germline [eg, buccal swab or other germline tissue sample] and donor testing, twin zygosity testing or maternal cell contamination of fetal cells (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
CYPZ	CYP21A2 Gene, Full Gene Analysis	94197-1

Result ID	Test Result Name	Result LOINC® Value
37488	Result Summary	50397-9
37489	Result	82939-0
37490	Interpretation	69047-9
37491	Additional Information	48767-8
37492	Specimen	31208-2
37493	Source	31208-2

37494	Released By	18771-6