MAYO CLINIC LABORATORIES

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

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

Known/familial variant analysis for *CYP21A2,* as due to the complexity of the *CYP21A2* locus, site specific testing for known/familial variants is not offered for this gene

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 gene sequencing and multiplex ligation-dependent probe amplification 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 at an additional charge.

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

For any prenatal specimen that is received, maternal cell contamination studies will be added.

Special Instructions

- Informed Consent for Genetic Testing
- CYP21A2 Gene Testing for Congenital Adrenal Hyperplasia Patient Information
- Informed Consent for Genetic Testing (Spanish)

Highlights

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

Full gene sequencing and multiplex ligation-dependent probe amplification 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)

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.

Additional Testing Requirements

All prenatal specimens must be accompanied by a maternal blood specimen; order MATCC / Maternal Cell Contamination, Molecular Analysis on the maternal specimen.

Shipping Instructions

Specimen preferred to arrive within 96 hours of collection.

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 providers 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 testing patients who have received a bone marrow transplant, call 800-533-1710 for instructions.

Submit only 1 of the following specimens:

Specimen Type: Whole blood
Container/Tube:
Preferred: Lavender top (EDTA)
Specimen Volume: 3 mL
Collection Instructions:
1. Invert several times to mix blood.
2. Send specimen in original tube. **Do not** aliquot.
Specimen Stability Information: Ambient (preferred) 4 days/Refrigerated 14 days

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.

Specimen Type: Amniotic fluid
Container/Tube: Amniotic fluid container
Specimen Volume: 20 mL
Specimen Stability Information: Refrigerated (preferred)/Ambient
Additional information:
1. 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.
2. 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
Additional Information:
1. 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.
2. All prenatal specimens must be accompanied by a maternal blood specimen; order MATCC / Maternal Cell Contamination, Molecular Analysis, Varies on the maternal specimen.

Acceptable
Specimen Type: Confluent cultured cells
Container/Tube: T-25 flask
Specimen Volume: 2 Flasks
Collection Instructions: Submit confluent cultured cells from another laboratory.

Specimen Stability Information: Ambient (preferred)/Refrigerated
Additional Information: 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.

Specimen Minimum Volume

Amniotic Fluid: 10 mL; Blood: 1 mL; Chorionic villi: 5 mg

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	Ambient (preferred)		
	Frozen		
	Refrigerated		

Clinical & Interpretive

Clinical Information

Congenital adrenal hyperplasia (CAH), with an incidence rate of 1 in 10,000 to 18,000 live births, is one of the most common inherited syndromes. The condition 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 within the highly recombinant human histocompatibility complex locus. 21-hydroxylase deficient CAH is inherited in an autosomal recessive pattern and has a spectrum of clinical phenotypes depending upon residual enzyme activity. Excessive adrenal androgen biosynthesis results in varying degrees of virilization. If there is some residual enzyme activity, a non-classical phenotype results, with signs of hyperandrogenism typically starting in later childhood or adolescence. Individuals with severe enzyme deficiency have classical CAH, with prenatal onset of virilization. Classical CAH is subdivided into simple-virilizing (minimal residual enzyme activity) and salt-wasting (no 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 screened for 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 many non-classical cases, which may present later in childhood or adolescence and require more extensive steroid hormone profiling, including testing before and after adrenal stimulation with adrenocorticotropic hormone (ACTH)-1-24.

For these reasons, genetic diagnosis plays an important ancillary role in both classical and nonclassical cases. In addition, the high carrier frequency (approximately 1 in 50) for *CYP21A2* variant makes genetic diagnosis important for genetic counseling. 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, partial or complex rearrangements (with or without accompanying gene duplication events), which lead to reciprocal exchanges between gene and pseudogene, can present severe diagnostic challenges. Comprehensive genetic testing strategies must therefore allow accurate assessment of most, or all, known rearrangements and variants, as well as unequivocal determination of whether the observed changes are located within a potentially transcriptionally active genetic segment. Testing of additional family members is often needed for clarification of genetic test results.

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 alterations (ie, polymorphisms) in primer binding sites can lead to selective allelic drop-out, which can lead to false-negative or false-positive diagnosis.

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.

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 May;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

Performance

Method Description

A combined testing approach involving polymerase chain reaction (PCR) amplification, bi-directional sequence analysis, and multiplex ligation-dependent probe amplification (MLPA) 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 and 3'-untranslated regions, 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.

MLPA is performed to determine the copy number of the 5'- and 3'-regions of the *CYP21A2* gene and the CYP21A1P pseudogene. Quantification and comparison of results is used to determine the copy number of the *CYP21A2* gene, the CYP21A1P pseudogene, the CYP21A2/CYP21A1P and CYP21A1P/CYP21A2 hybrids. Correlation of results from PCR, bi-directional sequencing, and MLPA 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 might 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: 2 weeks (if available) Extracted DNA: 3 months

Performing Laboratory Location
Rochester

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)
81479 (if appropriate for government payers)

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