MAYO CLINIC LABORATORIES

CYP21A2 Gene Analysis: Frequently Asked Questions

Due to the complexity of the *CYP21A2* gene locus, clinicians and other healthcare professionals may encounter challenges in testing strategy, risk assessment, and clinical interpretation of results. This document provides guidance on some of the most common questions our laboratory receives related to *CYP21A2* molecular testing: test ID CYPZ / *CYP21A2* Gene, Full Gene Analysis. If you need additional assistance, contact Mayo Clinic Laboratories at 800-533-1710 and ask to speak with a laboratory genetic counselor.

Section 1: Ordering Guidance

1.1 Do you offer targeted testing for known familial variants?

No, only full gene analysis is offered due to the complexities of *CYP21A2*: see test ID CYPZ / *CYP21A2* Gene, Full Gene Analysis. Even if *CYP21A2* variants were previously detected in the family, the test methodologies used in CYPZ allow full characterization of the patient's genotype, including detection of any hybrids that can be missed on traditional carrier screening panels. By performing full gene analysis, our laboratory has detected additional, previously undetected, reportable variants in past cases, and in certain instances, has been able to determine the phasing of variants in patients with complex genotypes.

1.2 Are you able to accept prenatal specimens for CYPZ / CYP21A2 Gene, Full Gene Analysis?

Yes, we accept a variety of prenatal specimen types for CYPZ testing, including chorionic villi (CVS), amniotic fluid, cultured chorionic villi, and cultured amniocytes. Because this assay requires a large amount of high-quality DNA, which typically cannot be obtained from direct prenatal extractions, any uncultured prenatal specimen received will be cultured at an additional charge. For any cultured specimen, a culturing code (either CULFB for cultured fibroblasts or CULAF for cultured amniocytes) will be added, as our laboratory maintains cultures throughout the CYPZ testing process.

1.3 Can you perform prenatal testing when one or both parents have a p.Gln319*+ CYP21A2 full gene duplication carrier screening result?

Yes, we can perform prenatal testing in these cases. Additionally, in most cases, we are able to provide further resolution of the *CYP21A2* genotype in the fetus. Importantly, we can generally determine if a fetus is expected to be affected with 21-hydroxylase deficient congenital adrenal hyperplasia (21-OHD CAH). However, if a fetus inherits the p.Gln319*+ *CYP21A2* full gene duplication, fetal carrier status may not be definitively determined, and the report would indicate "unlikely carrier" (see section 2.2). Section 2 includes additional details on the p.Gln319*+ *CYP21A2* full gene duplication result.

1.4 Prenatal ordering logistics

For specimen requirements, see the Specimen section in our test catalog.

A maternal/gestational carrier blood specimen for test ID MATCC / Maternal Cell Contamination, Molecular Analysis is strongly recommended when submitting a prenatal specimen for CYPZ testing. Maternal cell contamination studies will be performed concurrently with fetal CYP21A2 full gene analysis.

Given the time-sensitive nature of prenatal testing and complexity of the *CYP21A2* locus, submitting blood specimens from both biological contributors (eg, the individuals providing the biological egg and sperm) is recommended when pursuing prenatal testing. These specimens will be used as controls. Having these control specimens available allows the laboratory, at our discretion, to perform additional testing to attempt to clarify unclear fetal *CYP21A2* results—such as determining the phase of the detected variants—without delaying fetal results. Once fetal testing is complete, parental control specimens will have testing canceled and will not have a report issued.

If you wish to send in control specimens, you must contact the genetic counseling team ahead of specimen shipment to ensure control specimens are flagged as controls upon specimen receipt. Control specimens received without an alert will undergo routine processing and billing with reportable results. For additional information or to discuss a prenatal case, contact us by phone at 800-533-1710.

Fetal specimen type	Fetal test IDs	Maternal test IDs	Paternal test IDs
Direct or cultured amniotic fluid	CYPZ MATCC CULAF	MATCC (blood, strongly recommended) CYPZ (blood, optional)	CYPZ (blood, optional)
Direct or cultured CVS	CYPZ MATCC CULFB	MATCC (blood, strongly recommended) CYPZ (blood, optional)	CYPZ (blood, optional)

Section 2: Resulting Guidance (including clarification of carrier status related to p.Gln319*+duplication result)

2.1 What results can I expect from test ID CYPZ?

CYPZ is a full gene analysis test. Copy number analysis is performed to detect the number of copies of *CYP21A2* and its highly homologous pseudogene, *CYP21A1P*. In addition, hybrid or chimera alleles (referred to as *CYP21A2::CYP21A1P* and *CYP21A1P::CYP21A2*) are also detected.

The *CYP21A2::CYP21A1P* hybrid is expected to produce an active protein, unless disease-causing variants impacting 21-hydroxylase activity are detected. An individual can have the *CYP21A2::CYP21A1P* hybrid and still have a negative result if no reportable variants are detected.

The hybrid *CYP21A1P::CYP21A2* is an inactive hybrid and the typical byproduct of the 30 kb deletion resulting from unequal crossover between *CYP21A2* and *CYP21A1P*.

The CYPZ report includes all results detected, including total number of copies of the gene, pseudogene, and any hybrids. A clinical interpretation of the result is also provided.

2.2 Can test ID CYPZ clarify the p.Gln319*+ *CYP21A2* full gene duplication result reported on carrier screening tests?

In most instances, the detection of heterozygous p.Gln319* and a full gene duplication of *CYP21A2* corresponds to a known "non-carrier haplotype." Test ID CYPZ cannot definitively phase this result; however, there are two benign single nucleotide polymorphisms (SNPs) in *CYP21A2* that have been reported to be associated with noncarrier status (eg, the p.Gln319* located in cis with an additional full copy of *CYP21A2* with no reportable variants) for individuals with this result. Since CYPZ includes full gene sequencing of *CYP21A2*, both "marker SNPs" are detected when present.

If both marker SNPs are present in an individual who is heterozygous for p.Gln319*, has three copies of *CYP21A2*, and no other reportable variants, our laboratory will interpret this result as being an "unlikely carrier."

However, although our testing is intended to be comprehensive and diagnostic, it is unlikely to provide definitive clarification of carrier status in individuals with the p.Gln319*+*CYP21A2* duplication genotype.

At this time, we do not have a published residual risk for individuals with this genotype.

2.3 Can testing of first-degree relatives (segregation/family studies) help to clarify a p.Gln319*+ *CYP21A2* full gene duplication result in the setting of carrier screening?

While rare instances exist where family studies do help to clarify carrier status in individuals with the p.Gln319*+duplication haplotype, in most instances, this is not the case.

The p.Gln319*+duplication allele is a common haplotype in the general population, and, based on our laboratory's experience, family studies using our testing methodology are unlikely to assist in confirming an individual's carrier status in the absence of additional reportable variants. If, for example, an individual is reported to be a carrier of a duplication of *CYP21A2*, heterozygous for p.Gln319*, and heterozygous for p.Val282Leu, family studies may be beneficial for that individual.

If there are questions related to the use of family studies for your specific patient, please contact Mayo Clinic Laboratories at 800-533-1710 and ask to speak to a laboratory genetic counselor.