Overview

Useful For

Providing information relevant to tamoxifen, codeine, and tramadol, as well as other medications metabolized by CYP2D6

Determining the exact genotype when other methods fail to generate this information or if genotype-phenotype discord is encountered clinically

Identifying exact genotyping when required (eg, drug trials, research protocols)

Identifying novel variants that may interfere with drug metabolism

Genetics Test Information

Testing is done in 2 tiers when needed. Tier 1 uses a PCR-based 5'-nuclease assay to determine the variants present. All samples also have copy number determined by PCR-based 5'-nuclease assay. Testing in tier 1 allows for the detection of all common *CYP2D6* variants (eg, *2, *3, *4, *5, *6, *7, *8, *9, *10, *17, *29, *35, *41) and rarer alleles such as *11, *12, *14A, *14B, and *15. Duplications and multiplications of alleles are also identified. Unitary and tandem *CYP2D7-2D6* (*13) alleles and *CYP2D6-2D7* (eg, *4N, *36, and *68) alleles can also be detected. Tier 2 testing involves sequencing using fluorescent dye-terminator chemistry and is only done if tier 1 testing results are ambiguous. Approximately 3% of samples require tier 2 testing.

Reflex Tests

Test ID	Reporting Name	Available Separately	Always Performed
2D6S1	CYP2D6 FULL GENE SEQUENCE	No, (Bill Only)	No
2D6S2	CYP2D6 GEN CYP2D6-2D7 HYBRID	No, (Bill Only)	No
2D6S3	CYP2D6 GEN CYP2D7-2D6 HYBRID	No, (Bill Only)	No
2D6S4	CYP2D6 NONDUPLICATED GENE	No, (Bill Only)	No
2D6S5	CYP2D6 5' GENE DUP/MLT	No, (Bill Only)	No
2D6S6	CYP2D6 3' GENE DUP/MLT	No, (Bill Only)	No

Testing Algorithm

Tier 2 testing will be performed only if an ambiguous phenotype is identified by tier 1 testing. The number of sequencing tests needed to determine the phenotype will vary depending on the tier 1 result.

See CYP2D6 Comprehensive Cascade Testing Algorithm in Special Instructions.

Special Instructions

- Informed Consent for Genetic Testing

- CYP2D6 Comprehensive Cascade Testing Algorithm
- Pharmacogenomic Associations Tables
- Multiple Genotype Test List
- Informed Consent for Genetic Testing (Spanish)

Method Name

Tier 1: Real Time Polymerase Chain Reaction (PCR)

Tier 2: Polymerase Chain Reaction (PCR) followed by DNA Sequence Analysis

NY State Available

Yes

Specimen

Specimen Type

Varies

Advisory Information

This test is not for use in assessing for autoimmune hepatitis. Autoantibodies for CYP2D6 enzyme are found in many cases of autoimmune hepatitis; order LKM / Liver/Kidney Microsome Type 1 Antibodies, Serum for autoimmune hepatitis assessment.

Testing is available as the single gene assay (this test) or as a part of a focused pharmacogenomics panel, which includes testing for the following genes: *CYP*s *1A2, 2C9, 2C19, 2D6, 3A4, 3A5, 4F2, SLCO1B1, VKORC1*. Order PGXFP / Focused Pharmacogenomics Panel if multiple pharmacogenomic genotype testing is desired.

Specimen Required

Multiple genotype tests can be performed on a single specimen after a single extraction. See Multiple Genotype Test List in Special Instructions for a list of tests that can be ordered together.

Submit only 1 of the following specimens:

Specimen Type: Whole blood

Container/Tube: Lavender top (EDTA)

Specimen Volume: 3 mL

Collection Instructions:

1. Invert several times to mix blood.

2. Send specimenin original tube.

Specimen Stability Information: Ambient (preferred) 9 days/Refrigerated 30 days

Specimen Type: Saliva

Patient Preparation: Patient should not eat, drink smoke, or chew gum 30 minutes prior to collection.

Supplies: Saliva Swab Collection Kit (T786)

Specimen Volume: 1 swab

Collection Instructions: Collect and send specimen per kit instructions.

Additional Information: Due to lower concentration of DNA yielded from saliva, testing cannot proceed to tier 2 sequencing and will stop after tier 1 testing is complete.

Specimen Stability Information: Ambient 30 days

Specimen Type: DNA

Container/Tube: 2 mL screw top tube

Specimen Volume: 100 mcL (microliters)

Collection Instructions:

1. The preferred volume is 100 mcL at a concentration of 75 ng/mcL.

2. Include concentration and volume on tube.

Specimen Stability Information: Frozen (preferred)/Ambient/Refrigerated

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 in Special Instructions:

-Informed Consent for Genetic Testing (T576)

-Informed Consent for Genetic Testing-Spanish (T826)

2. If not ordering electronically, complete, print, and send 1 of the following forms with the specimen:

-Cardiovascular Test Request (T724)

-Neurology Specialty Testing Client Test Request (T732)

-Therapeutics Test Request (T831)

Specimen Minimum Volume

Blood: 1 mL
Saliva: 1 swab

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 and Interpretive

Clinical Information

The cytochrome P450 (CYP) family of enzymes is a group of oxidative/dealkylating enzymes localized in the microsomes of many tissues including the intestines and liver. One of the CYP enzymes, CYP2D6, is wholly or partially responsible for the metabolism of many commonly prescribed drugs.

The *CYP2D6* gene is highly variable with over 100 named alleles. The gene may be deleted, duplicated, and multiplied, and can have multiple sequence variations. In addition, some individuals have genes that are hybrids of *CYP2D6* and the *CYP2D7* pseudogene. Some individuals have *CYP2D6* variants that result in synthesis of an enzyme with decreased or absent catalytic activity. These individuals may process CYP2D6-metabolized medications more slowly. *CYP2D6* duplications and multiplications involving active alleles may result in ultrarapid metabolism of CYP2D6-metabolized drugs. *CYP2D6* genotype results are used to predict ultrarapid, rapid, normal (extensive), intermediate to normal (extensive), intermediate, poor to intermediate, and poor metabolizer phenotypes.(See Table 1)

Table 1. Enzyme Activity of Individual Star Alleles

Enzyme Activity	**Examples of *CYP2D6* star alleles**
Normal (extensive) metabolism	**1, *35*
Intermediate to normal activity	**2A*
Decreased activity	**2, *9, *10, *14B, *17, *29,* and **41*
Negligible activity	**36*
No or null activity	**3, *4, *4N, *5, *6, *7, *8, *11, *12, *13, *14A, *15, *68*

CYP2D6 phenotype is predicted based upon the number of functional, partially functional, and nonfunctional alleles present in a sample.

Phenotyping was derived from the Human Cytochrome P450 (CYP) Allele Nomenclature Committee website and the PharmGKB website for the related Clinical Pharmacogenetics Implementation Consortium guidelines.

There are instances where a phenotype prediction is not categorical and, in these instances, a range of possible phenotypes will be given. It should be noted that other laboratories may use different phenotype prediction methods as there is no consensus on this at this time. However, the method used here represents the findings of the majority of literature available at this time. Individuals without a detectable gene alteration will have the predicted phenotype of an extensive drug metabolizer and are designated as *CYP2D6 *1/*1*.

Drugs that are metabolized through CYP2D6 may require dosage adjustment based on the individual patient's genotype. Patients who are poor metabolizers may require lower than usual doses to achieve optimal response in the case of drugs that are inactivated by the CYP2D6 enzyme and higher than usual doses in the case of drugs that are activated by CYP2D6 enzyme. Alternatively, patients who are ultrarapid metabolizers may benefit from increased doses in the case of drugs that are inactivated by CYP2D6 enzyme and lower doses in the case of drugs that are

activated by CYP2D6. In the absence of clear guidance from FDA on dosing for various metabolizer phenotypes, patients with either ultrarapid or poor metabolism may benefit by switching to comparable alternate medications not primarily metabolized by CYP2D6 or by therapeutic drug monitoring where applicable.

Overall, this test provides a comprehensive *CYP2D6* genotype result for patients, ensuring a more accurate phenotype prediction. This assay has clinical significance for patients taking or considering medications activated (eg, codeine, tramadol, and tamoxifen) or inactivated (eg, antidepressants and antipsychotics) by the CYP2D6 enzyme.

Sequential tier testing associated with this test will be initiated until the least ambiguous phenotype possible is determined.

Reference Values

A comprehensive interpretive report will be provided.

Interpretation

A comprehensive interpretive report will be provided that combines the results of all tier testing utilized to obtain the final genotype.

The genotype, with associated star alleles, is assigned using standard allelic nomenclature as published by the Pharmacogene Variation (PharmVar) Consortium.(1)

For the *CYP2D6* Copy Number Variation assay, the reportable copy number range is 0 to 4 copies for each of the *CYP2D6* region assessed.

Novel variants will be classified based on known, predicted, or possible effect on gene function and reported with interpretive comments detailing their potential or known significance.

For additional information regarding pharmacogenomic genes and their associated drugs, see Pharmacogenomic Associations Tables in Special Instructions. This resource also includes information regarding enzyme inhibitors and inducers, as well as potential alternate drug choices.

Cautions

Rare variants may be present that could lead to false-negative or false-positive results. If results obtained do not match the clinical findings (phenotype), additional testing should be considered.

Samples may contain donor DNA if obtained from patients who received heterologous blood transfusions or allogeneic blood or marrow transplantation. Results from samples obtained under these circumstances may not accurately reflect the recipient's genotype. For individuals who have received blood transfusions, the genotype usually reverts to that of the recipient within 6 weeks. For individuals who have received allogeneic blood or marrow transplantation, a pretransplant DNA specimen is recommended for testing.

CYP2D6 genetic test results in patients who have undergone liver transplantation may not accurately reflect the patient's *CYP2D6* status.

This method may not detect all *CYP2D6* variants that result in altered CYP2D6 activity. Therefore, absence of a detectable variant does not rule out the possibility that a patient has altered CYP2D6 metabolism due to other *CYP2D6* variants that cannot be detected with this method. Furthermore, when 2 or more variants are identified, the cis-/trans- status (whether the variants are on the same or opposite chromosomes) is not always known.

A complicating factor in correlating *CYP2D6* genotype with phenotype is that many drugs or their metabolites are inhibitors of CYP2D6 catalytic activity. Selective-serotonin reuptake inhibitors (SSRIs), as well as some tricyclic antidepressants (TCAs) and other drugs, may reduce CYP2D6 catalytic activity. Patients in all metabolizer categories, except poor metabolizer, may have CYP2D6 enzyme activity inhibited by a variety of medications or their metabolites. Consequently, an individual may require a lower medication dose than predicted by genotyping alone. It is important to interpret the results of testing in the context of other coadministered drugs.

CYP2D6 alleles with decreased function may metabolize different drugs at different rates, ranging from normal to poor, but the literature on this is incomplete at this time.

This test is not designed to provide specific dosing or drug selection recommendations and is to be used as an aid to clinical decision making only. Drug-label guidance should be used when dosing patients with medications regardless of the predicted phenotype.

Clinical Reference

1. Pharmacogene Variation Consortium database. Accessed 08/27/2018. Available at www.pharmvar.org/gene/CYP2D6

2. Black JL, Walker DL, O'Kane DJ, Harmandayan M: Frequency of undetected *CYP2D6* hybrid genes in clinical samples: impact on phenotype prediction. Drug Metab Dispos 2012;40(1):111-119

3. Goetz MP, Rae M, Suman VJ, et al: Pharmacogenetics of tamoxifen biotransformation is associated with clinical outcomes of efficacy and hot flashes. J Clin Oncol 2005;23:9312-9318

4. Kircheiner J, Nickchen K, Bauer M, et al: Pharmacogenetics of antidepressants and antipsychotics: the contribution of allelic variations to the phenotype of drug response. Mol Psychiatry 2004;9:442-473

5. Crews KR, Gaedigk A, Dunnenberger HM, et al: Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for codeine therapy in the context of cytochrome P450 2D6 (*CYP2D6*) genotype. Clin Pharmacol Ther 2011 Feb;91(2):321-326

6. Hicks JK, Swen JJ, Thorn CF, et al: Clinical Pharmacogenetics Implementation Consortium guideline for *CYP2D6* and *CYP2C19* genotypes and dosing of tricyclic antidepressants. Clin Pharmacol Ther 2013 May:93(5):402-408

Performance

Method Description

Genomic DNA is extracted from whole blood or saliva.

Genotype Assay (Tier 1):

Genotyping is performed using a PCR-based 5'-nuclease assay. Fluorescently labeled detection probes anneal to the target DNA. PCR is used to amplify the section of DNA that contains the variant. If the detection probe is an exact match to the target DNA, the 5'-nuclease polymerase degrades the probe, the reporter dye is released from the effects of the quencher dye, and a fluorescent signal is detected. Genotypes are assigned based on the allele-specific fluorescent signals that are detected.(User Guide: TaqMan SNP Genotyping Assay, Applied Biosystems, Revision A.0 January 2014)

Copy Number Assay (Tier 1):

This assay utilizes a duplex real-time PCR, which includes 1 copy number probe and a reference assay per reaction.

Each copy number probe detects the genomic sequence of interest and the reference assay detects a sequence that is known to be present in 2 copies in a diploid genome. Relative quantitation is used to determine the relative copy number of the target of interest in a genomic DNA (gDNA) sample normalized to 10 ng/mcL for each probe. Each probe is normalized to the known copy number of the reference sequence, and compared to a calibrator sample with known copies of the target sequence included with each run.(Package insert: Taqman Copy Number Assays Revision D, Applied Biosystems, Carlsbad, CA)

Sequencing Assays (Tier 2):

The *CYP2D6* allele of interest is amplified by PCR. The PCR product is then purified and sequenced in both directions using fluorescent dye-terminator chemistry. Sequencing products are separated on an automated sequencer and trace files analyzed for variations in the exons and intron/exon boundaries of all 9 exons using mutation detection software and visual inspection.(Unpublished Mayo method)

PDF Report

No

Day(s) and Time(s) Test Performed

Monday through Thursday; 8 a.m.

Analytic Time

3 days (not reported Saturday or Sunday)

Maximum Laboratory Time

16 days

Specimen Retention Time

Whole Blood/Saliva swab: 2 weeks Extracted DNA: 2 months

Performing Laboratory Location

Rochester

Fees and 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 Regional Manager. 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. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information

0070U

0071U-0076U (if appropriate)

LOINC® Information

Test Definition: 2D6CV

CYP2D6 Genotype Cascade

Test ID	Test Order Name	Order LOINC Value
2D6CV	CYP2D6 Genotype Cascade	47403-1

Result ID	Test Result Name	Result LOINC Value
BA0113	CYP2D6 Genotype	40425-1
BA0114	CYP2D6 Phenotype	79715-9
BA0115	Interpretation	69047-9
BA0116	Additional Information	48767-8
BA0195	Method	49549-9
BA0196	Disclaimer	62364-5
BA0117	Reviewed by	18771-6