MAYO CLINIC LABORATORIES

Test Definition: TPNUQ

Thiopurine Methyltransferase (TPMT) and Nudix Hydrolase (NUDT15) Genotyping, Varies

Overview

Useful For

Predicting potential for toxicity to thiopurine drugs (6-mercaptopurine, 6-thioguanine, and azathioprine)

Testing Algorithm

For more information see Ulcerative Colitis and Crohn Disease Therapeutic Drug Monitoring Algorithm.

Special Instructions

- Informed Consent for Genetic Testing
- Pharmacogenomic Association Tables
- Multiple Genotype Test List
- Informed Consent for Genetic Testing (Spanish)
- Ulcerative Colitis and Crohn Disease Therapeutic Drug Monitoring Algorithm

Highlights

This test includes genotyping of *TPMT* and *NUDT15*, both of which affect metabolism of thiopurine drugs.

Method Name

Real-Time Polymerase Chain Reaction (PCR) With Allelic Discrimination Analysis

NY State Available

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

For thiopurine methyltransferase (TPMT) enzyme activity testing, order TPMT3 / Thiopurine Methyltransferase Activity Profile, Erythrocytes; however, this test should also be ordered because TPMT enzyme activity testing cannot detect variants in *NUDT15*, which also impact thiopurine metabolism.

Specimen Required

Multiple genotype tests can be performed on a single specimen after a single extraction. See Multiple Genotype Test List 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 whole blood specimen in original tube. **Do not aliquot.**
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.
Specimen Stability Information: Ambient 30 days

Specimen Type: Extracted DNA
Container/Tube: 2-mL screw top tube
Specimen Volume: 100 mcL
Collection Instructions:
1. The preferred volume is 100 mcL at a concentration of 50 ng/mcL.
2. Provide concentration of DNA 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:
-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:
-Neurology Specialty Testing Client Test Request (T732)
-Gastroenterology and Hepatology Test Request (T728)
-Therapeutics Test Request (T831)

Specimen Minimum Volume

Blood: 0.4 mL
Saliva, extracted DNA: 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

The thiopurine drugs are purine antimetabolites that are useful in the treatment of acute lymphoblastic leukemia, autoimmune disorders (eg, Crohn disease, rheumatoid arthritis), and organ transplant recipients. The thiopurine drugs, 6-mercaptopurine, 6-thioguanine, and azathioprine are prodrugs that require intracellular activation to 6-thioguanine nucleotides (6-TGN). This activation is catalyzed by multiple enzymes. The cytotoxic effects of thiopurine drugs are achieved mainly through incorporation of 6-TGN into DNA and RNA. The pathway that leads to synthesis of active cytotoxic 6-TGN is in competition with inactivation pathways catalyzed by thiopurine methyltransferase (TPMT). Evaluation of this pathway is important because the level of 6-TGN measured in red blood cells have been correlated with both thiopurine therapeutic efficacy and toxicity such as myelosuppression.

TPMT activity is inherited as a monogenic codominant trait, and variable TPMT activity is associated with *TPMT* genetic variants. The distribution of TPMT activity in red blood cells is trimodal in the White population, with approximately 0.3% of people having deficient (undetectable) TPMT activity, 11% low (intermediate) activity, and 89% normal TPMT activity. The allele for normal TPMT activity (wild type) has been designated *TPMT*1*. Four *TPMT* alleles, comprised of a combination of 3 different single-nucleotide variants, account for the majority of inactivating alleles in some ethnicities, including whites: *TPMT*2*, *TPMT*3A*, and *TPMT*3C*. Less frequently occurring *TPMT* alleles *TPMT*4*, *TPMT*5*, *TPMT*8*, and *TPMT*12* also have been implicated as deficiency alleles. If no *TPMT* variant alleles are detected by this assay, the most likely genotype is that of *TPMT*1/*1*, although the presence of other rarer alleles cannot be excluded.

Nudix hydrolase (NUDT15) is thought to dephosphorylate the active metabolites of thiopurines, TGTP, and TdGTP, which prevents their incorporation into DNA and decreases their cytotoxic effects. Genetic variants in *NUDT15* that decrease this activity are strongly associated with thiopurine-related myelosuppression. NUDT15 deficiency is most common among East Asian (22.6%), South Asian (13.6%), and Native American populations (12.5%-21.2%). Studies in other populations are ongoing. This test evaluates variants associated with *NUDT15*2, NUDT15*3, NUDT15*4,* and *NUDT15 *5*. If no *NUDT15* variant alleles are detected by this assay, the most likely genotype is that of *NUDT15*1/*1*, although the presence of other rarer alleles cannot be excluded. Individuals with variants in both *TPMT* and *NUDT15* have been identified and were significantly more sensitive to mercaptopurine than individuals heterozygous for a variant in only one gene. Integration of both *TPMT* and *NUDT15* testing may allow for more accurate prediction of thiopurine-related toxicity risk to guide dosing, particularly among patients from diverse populations.

Table 1. TPMT Enzyme Activity of Individual Star Alleles

***TPMT* allele**	**cDNA nucleotide change (NM_000367.4)**	**Amino acid change**	**Effect on enzyme metabolism**
**1*	None (wild type)	None (wild type)	Normal function
**2*	c.238G>C	p.Ala80Pro (p.A80P)	No activity
**3A*	c.460G>A and c.719A>G	p.Ala154Thr (p.A154T) and p.Tyr240Cys (p.Y240C)	No activity
**3B*	c.460G>A	p.Ala154Thr (p.A154T)	No activity
**3C*	c.719A>G	p.Tyr240Cys (p.Y240C)	No activity
**4*	c.626-1G>A	Not applicable, splice site	No activity
**5*	c.146T>C	p.Leu49Ser (p.L49S)	No activity
**8*	c.644G>A	p.Arg215His (p.R215H)	Reduced activity
**12*	c.374C>T	p.Ser125Leu (p.S125L)	Reduced activity

Table 2. NUDT15 Enzyme Activity of Individual Star Alleles

***NUDT15* allele**	**cDNA nucleotide change (NM_018283.3)**	**Amino acid change**	**Effect on enzyme metabolism**
**1*	None (wild type)	None (wild type)	Normal activity
**2* or **3*	c.415C>T	p.Arg139Cys (p.R139C)	No activity
**4*	c.416G>A	p.Arg139His (p.R139H)	No activity
**5*	c.52G>A	p.Val18Ile (p.V18I)	No activity

The US Food and Drug Administration, the Clinical Pharmacogenetics Implementation Consortium, and some professional societies recommend consideration of *TPMT* and *NUDT15* genotype testing or TPMT enzyme activity testing along with *NUDT15* genotype testing prior to the initiation of therapy with thiopurine drugs. There is substantial evidence linking *TPMT* and *NUDT15* genotypes to phenotypic variability. Dose adjustments based upon *TPMT* and *NUDT15* genotypes have reduced thiopurine-induced adverse effects without compromising desired antitumor and immunosuppressive therapeutic effects in several clinical settings.

Genotyping is not impacted by other medications known to inhibit TPMT activity. Complementary clinical testing is available to measure TPMT enzymatic activity in erythrocytes (TPMT3 / Thiopurine Methyltransferase Activity Profile, Erythrocytes) if the clinician wants to check for lower TPMT enzyme activity, regardless of cause. Testing for TPMT enzyme activity is not impacted by variants in *NUDT15*.

Reference Values

An interpretive report will be provided.

Interpretation

An interpretive report will be provided.

The *TPMT* genotype, with associated star alleles, is assigned using standard allelic nomenclature as published by the TPMT Nomenclature Committee.(1) *NUDT15* genotype and associated star alleles are as described by Moriyama et al.(2) and catalogued in the Pharmacogene Variation Consortium (www.pharmvar.org).

For additional information regarding pharmacogenomic genes and their associated drugs, see the Pharmacogenomics Associations Tables. 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 no *TPMT* variant alleles are detected by this assay the most likely genotype is that of *TPMT*1/*1*, although the presence of other rarer alleles cannot be excluded. In addition, if no *NUDT15* variant alleles are detected by this assay, the most likely genotype is that of *NUDT15*1/*1*, although the presence of other rarer alleles cannot be excluded.

If genotype results obtained do not match the clinical findings, additional testing should be considered for thiopurine methyltransferase enzyme activity (TPMT3 / Thiopurine Methyltransferase Activity Profile, Erythrocytes). A corresponding activity assay for NUDT15 is not currently available.

Samples may contain donor DNA if obtained from patients who received non-leukoreduced blood transfusions or allogeneic hematopoietic stem cell 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 hematopoietic stem cell transplantation, a pretransplant DNA specimen is recommended for testing.

The results do not rule out the possibility that a patient harbors another variant in *TPMT, NUDT15,* or another gene that can impact drug response or side effects. These genotyping procedures will not distinguish between heterozygous *TPMT*3A* from the rare *TPMT*3B/*3C*, which has an estimated frequency of 1:120,890. This rare genotype is associated with low enzyme activity. Enzyme activity evaluation is necessary to definitively identify this rare genotype (TPMT3 / Thiopurine Methyltransferase Activity Profile, Erythrocytes).

This test will not detect all *TPMT* or *NUDT15* genetic variants. A negative result does not rule out the possibility of toxicity if thiopurines are used, since multiple factors (eg, other genetic factors, drug-drug interactions) are known to play a role. Co-prescription of allopurinol might inhibit TPMT activity. Other drugs that have been shown to inhibit TPMT activity include naproxen, ibuprofen, ketoprofen, furosemide, sulfasalazine, mesalamine, olsalazine, mefenamic acid, thiazide diuretics, and benzoic acid inhibitors.

Clinical Reference

1. TPMT nomenclature committee (TPMT Alleles): Table of TPMT Alleles. Linkoping University; Updated May 2019. Accessed October 6, 2022. Available at https://liu.se/en/research/tpmt-nomenclature-committee
2. Moriyama T, Nishii R, Perez-Andreu V, et al: NUDT15 polymorphisms alter thiopurine metabolism and hematopoietic toxicity. Nat Genet. 2016 Apr;48(4):367-373. doi: 10.1038/ng.3508
3. Appell ML, Berg J, Duley J, et al: Nomenclature for alleles of the thiopurine methyltransferase gene. Pharmacogenet Genomics. 2013 Apr;23(4):242-248. doi:1 0.1097/FPC.0b013e32835f1cc0
4. Nguyen CM, Mendes MA, Ma JD: Thiopurine methyltransferase (TPMT) genotyping to predict myelosuppression risk. PLoS Curr. 2011 May;3:RRN1236. doi: 10.1371/currents.RRN1236
5. Relling MV, Schwab M, Whirl-Carrillo M, et al: Clinical Pharmacogenetics Implementation Consortium guideline for thiopurine dosing based on TPMT and NUDT15 genotypes: 2018 Update. Clin Pharmacol Ther. 2019 May;105(5):1095-1105. doi: 10.1002/cpt.1304
6. Weinshilboum R: Thiopurine pharmacogenetics: clinical and molecular studies of thiopurine methyltransferase. Drug Metab Dispos. 2001 Apr;29(4 Pt 2):601-605
7. Zaza G, Cheok M, Krynetskaia N, et al: Thiopurine pathway. Pharmacogenet Genomics. 2010 Sept;20(9):573-574. doi: 10.1097/FPC.0b013e328334338f

Performance

Method Description

Genomic DNA is extracted from whole blood or saliva. Genotyping for the *TPMT* and *NUDT15* alleles is performed using a polymerase chain reaction (PCR)-based 5'-nuclease assay. Fluorescently labeled detection probes anneal to the target DNA. PCR is used to amplify the segment of DNA that contains the polymorphism. 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.(Unpublished Mayo method)

PDF Report

No

Day(s) Performed

Monday through Friday

Report Available
2 to 4 days

Specimen Retention Time
Whole blood/Saliva: 2 weeks; Extracted DNA: 2 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
0034U

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
TPNUQ	TPMT and NUDT15 Genotype, V	93193-1

Result ID	Test Result Name	Result LOINC® Value
610159	TPMT Genotype	41048-0
610160	TPMT Phenotype	79713-4
610161	NUDT15 Genotype	93194-9
610162	NUDT15 Phenotype	93195-6
610163	Interpretation	69047-9
610164	Additional Information	48767-8
610165	Method	85069-3
610166	Disclaimer	62364-5
610167	Reviewed by	18771-6