Overview

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

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

Special Instructions
- Informed Consent for Genetic Testing
- Pharmacogenomic Associations Tables
- Multiple Genotype Test List
- Informed Consent for Genetic Testing (Spanish)

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 (TPMT) Activity Profile, Erythrocytes.

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 specimen in 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: One swab

Collection Instructions: Collect and send specimen per kit instructions.

Specimen Stability Information: Ambient 30 days

Specimen Type: 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. 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:

   - Neurology Specialty Testing Client Test Request (T732)
   - Gastroenterology and Hepatology Client Test Request (T728)

   - Therapeutics Test Request (T831)

Specimen Minimum Volume

Blood: 0.4 mL
Saliva: 1 swab

Reject Due To

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

Specimen Stability Information
Test Definition: TPNUV
TPMT and NUDT15 Genotype

Clinical and 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-MP), 6-thioguanine (6-TG), and azathioprine (AZA) 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 Caucasians, 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 substitutions (SNP), account for the majority of inactivating alleles in some ethnicities, including Caucasians: TPMT*2, TPMT*3A, TPMT*3B, 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 myelosuppresion. NUDT deficiency is most common among East Asians (22.6%), followed by South Asians (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 with variants in only 1 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>
<thead>
<tr>
<th>TPMT Allele</th>
<th>cDNA Nucleotide Change</th>
<th>Amino Acid Change</th>
<th>Effect on Enzyme Metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>*1</td>
<td>None (wild type)</td>
<td>None (wild type)</td>
<td>Normal function</td>
</tr>
<tr>
<td>*2</td>
<td>c.238G&gt;C</td>
<td>p.Ala80Pro (p.A80P)</td>
<td>No activity</td>
</tr>
<tr>
<td>*3A</td>
<td>c.460G&gt;A and c.719A&gt;G</td>
<td>p.Ala154Thr (p.A154T) and p.Tyr240Cys (p.Y240C)</td>
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</tr>
<tr>
<td>*3B</td>
<td>c.460G&gt;A</td>
<td>p.Ala154Thr (p.A154T)</td>
<td>No activity</td>
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Test Definition: TPNUV
TPMT and NUDT15 Genotype

<table>
<thead>
<tr>
<th>Allele</th>
<th>cDNA Nucleotide Change</th>
<th>Amino Acid Change</th>
<th>Effect on Enzyme Metabolism</th>
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<tbody>
<tr>
<td>*3C</td>
<td>c.719A&gt;G</td>
<td>p.Tyr240Cys (p.Y240C)</td>
<td>No activity</td>
</tr>
<tr>
<td>*4</td>
<td>c.626-1G&gt;A</td>
<td>Not applicable, splice site</td>
<td>No activity</td>
</tr>
<tr>
<td>*5</td>
<td>c.146T&gt;C</td>
<td>p.Leu49Ser (p.L49S)</td>
<td>No activity</td>
</tr>
<tr>
<td>*12</td>
<td>c.374C&gt;T</td>
<td>p.Ser125Leu (p.S125L)</td>
<td>Reduced activity</td>
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The US Food and Drug Administration, the Clinical Pharmacogenetics Implementation Consortium, and some professional societies recommend consideration of TPMT genotype or TPMT erythrocyte testing prior to the initiation of therapy with thiopurine drugs. There is substantial evidence linking TPMT genotype to phenotypic variability. Dose adjustments based upon TPMT genotype 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 (TPMT) Activity Profile, Erythrocytes) if the clinician wants to check for lower TPMT enzyme activity, regardless of cause. Although there currently aren’t guidelines or professional society recommendations related to NUDT15 genotyping to guide thiopurine use, this practice is substantially supported by the literature. Testing for TPMT enzyme activity is not impacted by variants in NUDT15.

**NUDT15** Allele | cDNA Nucleotide Change | Amino Acid Change | Effect on Enzyme Metabolism |
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<tbody>
<tr>
<td>*1</td>
<td>None (wild type)</td>
<td>None (wild type)</td>
<td>Normal activity</td>
</tr>
<tr>
<td>*2 or *3</td>
<td>c.415C&gt;T</td>
<td>p.Arg139Cys (p.R139C)</td>
<td>No activity</td>
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<tr>
<td>*4</td>
<td>c.416G&gt;A</td>
<td>p.Arg139His (p.R139H)</td>
<td>No activity</td>
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<tr>
<td>*5</td>
<td>c.52G&gt;A</td>
<td>p.Val18Ile (p.V18I)</td>
<td>No activity</td>
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**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 Moriya et al.(2)

For additional information regarding pharmacogenomic genes and their associated drugs, see the Pharmacogenomics 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 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 [TPMT] Activity Profile, Erythrocytes). A corresponding activity assay for NUDT15 is not currently available.

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.

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 drug 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 [TPMT] 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. Coprescription of allopurinol might inhibit TPMT activity. 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. Available at www.imh.liu.se/tpmtalleles


**Performance**

**Method Description**

Genomic DNA is extracted from whole blood. Genotyping for the TPMT and NUDT15 alleles is performed using a 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. (Instruction manual: TaqMan SNP Genotyping Assay, Applied Biosystems Revision A.0 January 2014)
PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
1 to 4 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
0034U

LOINC® Information

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