

## Overview

### Useful For

Follow up for abnormal biochemical results suggestive of maple syrup urine disease (MSUD)

Establishing a molecular diagnosis for patients with MSUD

Identifying variants within genes known to be associated with MSUD, allowing for predictive testing of at-risk family members

### Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
CULFB	Fibroblast Culture for Genetic Test	Yes	No

### Genetics Test Information

This test utilizes next-generation sequencing to detect single nucleotide and copy number variants in 6 genes associated with maple syrup urine disease: *BCKDHA*, *BCKDHB*, *BCKDK*, *DBT*, *DLD*, *PPM1K*. See [Targeted Genes and Methodology Details for Maple Syrup Urine Disease Gene Panel](#) and Method Description for additional details.

Identification of a disease-causing variant may assist with diagnosis, prognosis, clinical management, familial screening, and genetic counseling for maple syrup urine disease.

### Testing Algorithm

For skin biopsy or cultured fibroblast specimens, fibroblast culture testing will be performed at an additional charge. If viable cells are not obtained, the client will be notified.

### Special Instructions

- [Molecular Genetics: Biochemical Disorders Patient Information](#)
- [Informed Consent for Genetic Testing](#)
- [Blood Spot Collection Card-Spanish Instructions](#)
- [Blood Spot Collection Card-Chinese Instructions](#)
- [Informed Consent for Genetic Testing \(Spanish\)](#)
- [Blood Spot Collection Instructions](#)
- [Targeted Genes and Methodology Details for Maple Syrup Urine Disease Gene Panel](#)

### Method Name

Sequence Capture and Targeted Next-Generation Sequencing (NGS) followed by Polymerase Chain Reaction (PCR) and Sanger Sequencing.

### NY State Available

Yes

## Specimen

### Specimen Type

Varies

### Ordering Guidance

The recommended first-tier screening tests for maple syrup urine disease (MSUD) are a combination of biochemical tests including quantitative plasma amino acids and urine organic acids. Order AAQP / Amino Acids, Quantitative, Plasma and OAU / Organic Acids Screen, Urine.

Customization of this panel and single gene analysis for any gene present on this panel is available. For more information see CGPH/ Custom Gene Panel, Hereditary, Generation Sequencing, Varies.

Targeted testing for familial variants (also called site-specific or known mutations testing) is available for the genes on this panel. See FMTT / Familial Variant, Targeted Testing, Varies. To obtain more information about this testing option, call 800-533-1710.

### Shipping Instructions

Specimen preferred to arrive within 96 hours of collection.

### Specimen Required

**Patient Preparation:** A previous bone marrow transplant from an allogenic donor will interfere with testing. For instructions for testing patients who have received a bone marrow transplant, call 800-533-1710.

### Submit only 1 of the following specimens:

**Specimen Type:** Whole blood

**Container/Tube:** Lavender top (EDTA) or yellow top (ACD)

**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) 4 days/Refrigerated 14 days

**Specimen Type:** Skin biopsy

**Supplies:** Fibroblast Biopsy Transport Media (T115)

**Container/Tube:** Sterile container with any standard cell culture media (eg, minimal essential media, RPMI 1640). The solution should be supplemented with 1% penicillin and streptomycin.

**Specimen Volume:** 4-mm punch

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

**Additional Information:** 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.

**Specimen Type:** Cultured fibroblast

**Container/Tube:** T-25 flask

**Specimen Volume:** 2 Flasks

**Collection Instructions:** Submit confluent cultured fibroblast cells from a skin biopsy from another laboratory. Cultured cells from a prenatal specimen will not be accepted.

**Specimen Stability Information:** Ambient (preferred)/Refrigerated (<24 hours)

**Additional Information:** 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.

**Specimen Type:** Blood spot

**Supplies:** Card-Blood Spot Collection (Filter Paper) (T493)

**Container/Tube:**

**Preferred:** Collection card (Whatman Protein Saver 903 Paper)

**Acceptable:** PerkinElmer 226 (formerly Ahlstrom 226) filter paper or blood spot collection card

**Specimen Volume:** 5 Blood spots

**Collection Instructions:**

1. An alternative blood collection option for a patient older than 1 year is a fingerstick. For detailed instructions, see [How to Collect Dried Blood Spot Samples](#).
2. Let blood dry on the filter paper at ambient temperature in a horizontal position for a minimum of 3 hours.
3. Do not expose specimen to heat or direct sunlight.
4. Do not stack wet specimens.
5. Keep specimen dry

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

**Additional Information:**

1. Due to lower concentration of DNA yielded from blood spot, it is possible that additional specimen may be required to complete testing.
2. For collection instructions, see [Blood Spot Collection Instructions](#)
3. For collection instructions in Spanish, see [Blood Spot Collection Card-Spanish Instructions](#) (T777)
4. For collection instructions in Chinese, see [Blood Spot Collection Card-Chinese Instructions](#) (T800)

**Specimen Type:** Saliva

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

**Supplies:**

DNA Saliva Kit High Yield (T1007)

Saliva Swab Collection Kit (T786)

**Container/Tube:**

**Preferred:** High-yield DNA saliva kit

**Acceptable:** Saliva swab

**Specimen Volume:** 1 Tube if using T1007 or 2 swabs if using T786

**Collection Instructions:** Collect and send specimen per kit instructions.

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

**Additional Information:** Saliva specimens are acceptable but not recommended. Due to lower quantity/quality of DNA yielded from saliva, some aspects of the test may not perform as well as DNA extracted from a whole blood sample. When applicable, specific gene regions that were unable to be interrogated will be noted in the report. Alternatively, additional specimen may be required to complete testing.

## 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:
  - [Informed Consent for Genetic Testing](#) (T576)
  - [Informed Consent for Genetic Testing \(Spanish\)](#) (T826)
- [2. Molecular Genetics: Biochemical Disorders Patient Information](#) (T527)
- If not ordering electronically, complete, print, and send a [Biochemical Genetics Test Request](#) (T798) with the specimen.

**Specimen Minimum Volume**

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**

Maple syrup urine disease (MSUD) is an inborn error of metabolism caused by the deficiency of the branched-chain ketoacid dehydrogenase (BCKDH) complex. The BCKDH complex is involved in the metabolism of the branched-chain amino acids (BCAA): isoleucine (Ile), leucine (Leu), and valine (Val).

MSUD can be divided into 5 phenotypes: classic, intermediate, intermittent, thiamine-responsive, and dihydrolipoyl dehydrogenase (E3)-deficient, depending on the clinical presentation and response to thiamine administration. Classic MSUD, the most common and most severe form, presents in newborns with feeding intolerance, failure to thrive, vomiting, lethargy, and maple syrup odor in urine and cerumen. If untreated, it progresses to irreversible intellectual disability, hyperactivity, failure to thrive, seizures, coma, cerebral edema, and possibly death.

Age of onset for individuals with non-classical forms of MSUD is variable, with some presenting with symptoms as early as 2 years of age. Symptoms include poor growth and feeding, irritability, and developmental delays. These patients can also experience severe metabolic intoxication and encephalopathy during periods of sufficient catabolic stress.

MSUD is a panethnic condition but is most prevalent in the Old Order Mennonite community in Lancaster, Pennsylvania with an incidence there of 1 in 760 live births. The incidence of MSUD is approximately 1 in 185,000 live births in the general population.

A comprehensive gene panel is a helpful tool to establish a diagnosis for patients with suggestive clinical and biochemical features given the broad clinical spectrum and genetic heterogeneity of MSUD. The BCKDH complex consists of 4 subunits (E1a, E1b, E2, E3), and this panel includes testing of the genes that encode each subunit (*BCKDHA* for E1a, *BCKDHB* for E1b, *DBT* for E2, and *DLD* for E3). In addition, *BCKDK* and *PPM1K* are also included, both of which impact the activity of the BCKDH complex. Disease-causing variants in both alleles of any of these genes result in

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disease.

The recommended first-tier tests to screen for MSUD is a combination of biochemical tests including quantitative plasma amino acids (AAQP / Amino Acids, Quantitative, Plasma) to measure BCAA levels and alloisoleucine and urine organic acids (OAU / Organic Acids Screen, Urine) to look for presence of toxic urine metabolites including 2-hydroxy-isovaleric acid and 2-oxo-isocaprioic acid.

Treatment of MSUD aims to normalize the concentration of BCAA by dietary restriction of these amino acids. Because BCAA belong to the essential amino acids, the dietary treatment requires frequent adjustment, which is accomplished by regular determination of BCAA and allo-isoleucine concentrations. Orthotopic liver transplantation has been successful and is an effective therapy for MSUD.

### **Reference Values**

An interpretive report will be provided.

### **Interpretation**

All detected alterations are evaluated according to American College of Medical Genetics and Genomics recommendations.(1) Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance.

### **Cautions**

#### Clinical Correlations:

Test results should be interpreted in the context of clinical findings, family history, and other laboratory data.

Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

If testing was performed because of a clinically significant family history, it is often useful to first test an affected family member. Detection of at least one reportable variant in an affected family member would allow for more informative testing of at-risk individuals.

To discuss the availability of additional testing options or for assistance in the interpretation of these results, contact the Mayo Clinic Laboratories genetic counselors at 800-533-1710.

#### Technical Limitations:

Next-generation sequencing may not detect all types of genomic variants. In rare cases, false-negative or false-positive results may occur. The depth of coverage may be variable for some target regions; assay performance below the minimum acceptable criteria or for failed regions will be noted. Given these limitations, negative results do not rule out the diagnosis of a genetic disorder. If a specific clinical disorder is suspected, evaluation by alternative methods can be considered.

There may be regions of genes that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences. Confirmation of select reportable variants will be performed by alternate methodologies based on internal laboratory criteria.

This test is validated to detect 95% of deletions up to 75 base pairs (bp) and insertions up to 47 bp. Deletions-insertions (delins) of 40 or more bp, including mobile element insertions, may be less reliably detected than smaller delins.

**Deletion/Duplication Analysis:**

This analysis targets single and multi-exon deletions/duplications; however, in some instances single exon resolution cannot be achieved due to isolated reduction in sequence coverage or inherent genomic complexity. Balanced structural rearrangements (such as translocations and inversions) may not be detected.

This test is not designed to detect low levels of mosaicism or to differentiate between somatic and germline variants. If there is a possibility that any detected variant is somatic, additional testing may be necessary to clarify the significance of results.

Genes may be added or removed based on updated clinical relevance. For detailed information regarding gene-specific performance and technical limitations, see Method Description or contact a laboratory genetic counselor.

If the patient has had an allogeneic hematopoietic stem cell transplant or a recent heterologous blood transfusion, results may be inaccurate due to the presence of donor DNA. Call Mayo Clinic Laboratories for instructions for testing patients who have received a bone marrow transplant.

**Reclassification of Variants:**

Currently, it is not standard practice for the laboratory to systematically review previously classified variants on a regular basis. The laboratory encourages healthcare providers to contact the laboratory at any time to learn how the classification of a particular variant may have changed over time. Due to broadening genetic knowledge, it is possible that the laboratory may discover new information of relevance to the patient. Should that occur, the laboratory may issue an amended report.

**Variant Evaluation:**

Evaluation and categorization of variants is performed using published American College of Medical Genetics and Genomics and the Association for Molecular Pathology recommendations as a guideline.(1) Other gene-specific guidelines may also be considered. Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance. Variants classified as benign or likely benign are not reported.

Multiple in silico evaluation tools may be used to assist in the interpretation of these results. The accuracy of predictions made by in silico evaluation tools is highly dependent upon the data available for a given gene, and periodic updates to these tools may cause predictions to change over time. Results from in silico evaluation tools should be interpreted with caution and professional clinical judgment.

Rarely, incidental or secondary findings may implicate another predisposition or presence of active disease. These findings will be carefully reviewed to determine whether they will be reported.

**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;17(5):405-424.
2. Chuang DT, Shih VE, Wynn RM. Maple syrup urine disease (branched-chain ketoaciduria). In: Valle D, Antonarakis S, Ballabio A, Beaudet A, Mitchell GA. eds. *The Online Metabolic and Molecular Bases of Inherited Disease*. McGraw-Hill

Education; 2019. Accessed March 8, 2024. Available at

<https://ommbid.mhmedical.com/content.aspx?sectionid=225084607&bookid=2709#225084691>

3. Frazier DM, Allgeier C, Horner C, et al. Nutrition management guideline for maple syrup urine disease: an evidence- and consensus-based approach. *Mol Genet Metab.* 2014;112(3):210-217

4. Strauss KA, Puffenberger EG, Morton DH. Maple Syrup Urine Disease. In: Adam MP, Ardinger HH, Pagon RA, et al, eds. *GeneReviews* [Internet]. University of Washington, Seattle; 2006. Updated April 23, 2020. Accessed March 8, 2024. Available at [www.ncbi.nlm.nih.gov/books/NBK1319](http://www.ncbi.nlm.nih.gov/books/NBK1319)

5. Diaz VM, Camarena C, de la Vega A, et al. Liver transplantation for classical maple syrup urine disease: long-term follow-up. *J Pediatr Gastroenterol Nutr.* 2014;59(5):636-639

## Performance

### Method Description

Next-generation sequencing (NGS) and/or Sanger sequencing are performed to test for the presence of variants in coding regions and intron/exon boundaries of the genes analyzed, as well as some other regions that have known disease-causing variants. The human genome reference GRCh37/hg19 build was used for sequence read alignment. At least 99% of the bases are covered at a read depth over 30X. Sensitivity is estimated to be over 99% for single nucleotide variants, over 94% for deletions-insertions (delins) less than 40 base pairs (bp), and over 95% for deletions up to 75 bp and insertions up to 47 bp. NGS and/or a polymerase chain reaction-based quantitative method is performed to test for the presence of deletions and duplications in the genes analyzed.

There may be regions of genes that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences. See [Targeted Genes and Methodology Details for Maple Syrup Urine Disease Gene Panel](#) for details regarding the targeted genes analyzed and the specific gene regions not routinely covered.(Unpublished Mayo method)

Genes analyzed: *BCKDHA*, *BCKDHB*, *BCKDK*, *DBT*, *DLD*, *PPM1K*

### PDF Report

Supplemental

### Day(s) Performed

Varies

### Report Available

14 to 21 days

### Specimen Retention Time

Whole blood: 2 weeks (if available); Extracted DNA: 3 months; Blood spots/Saliva: 1 month

### Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Main Campus

## 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

81406 x 3

81479

88233-Tissue culture, skin, solid tissue biopsy (if appropriate)

88240-Cryopreservation (if appropriate)

81479 (if appropriate for government payers)

### LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
MSUDP	MSUD Gene Panel	105355-2

Result ID	Test Result Name	Result LOINC® Value
608776	Test Description	62364-5
608777	Specimen	31208-2
608778	Source	31208-2
608779	Result Summary	50397-9
608780	Result	82939-0
608781	Interpretation	69047-9
608782	Resources	99622-3
608783	Additional Information	48767-8
608784	Method	85069-3
608785	Genes Analyzed	48018-6
608786	Disclaimer	62364-5
608787	Released By	18771-6