MAYO CLINIC LABORATORIES

Test Definition: ALLOI

Allo-isoleucine, BS

Overview

Useful For

Evaluation of newborn screening specimens that test positive for branched-chain amino acids elevations

Follow-up of patients with maple-syrup urine disease

Genetics Test Information

Second-tier test for abnormal newborn screen and follow-up of patients with maple syrup urine disease.

Special Instructions

- Blood Spot Collection Card-Spanish Instructions
- Blood Spot Collection Card-Chinese Instructions
- Blood Spot Collection Instructions

Method Name

Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

Portions of this test are covered by patents held by Quest Diagnostics

NY State Available

Yes

Specimen

Specimen Type

Whole blood

Specimen Required

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

Container/Tube: Local newborn screening card

Specimen Volume: 2 Blood spots

Collection Instructions:

1. Do not use device or capillary tube containing EDTA to collect specimen.
2. At least 1 spot should be complete and unpunched.
3. An alternative blood collection option for a patient older than 1 year of age is a fingerstick. See Dried Blood Spot Collection Tutorial for how to collect blood spots via fingerstick: https://vimeo.com/508490782 .
4. Include type of feeding information on the collection card.
5. Let blood dry on filter paper at ambient temperature in a horizontal position for a minimum of 3 hours.
6. Do not expose specimen to heat or direct sunlight.
7. Do not stack wet specimens.
8. Keep specimen dry.

Additional Information:

1. For collection instructions, see Blood Spot Collection Instructions
2. For collection instructions in Spanish, see Blood Spot Collection Card-Spanish Instructions (T777)
3. For collection instructions in Chinese, see Blood Spot Collection Card-Chinese Instructions (T800)

Forms

If not ordering electronically, complete, print, and send a Biochemical Genetics Test Request (T798) with the specimen.

Reject Due To

Blood spot shows layering	Reject
Serum rings	
Multiple applications	
Insufficient specimen	

Specimen Minimum Volume

1 Blood spot

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Whole blood	Ambient (preferred)		FILTER PAPER
	Frozen		FILTER PAPER
	Refrigerated		FILTER PAPER

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). Classic MSUD presents in the neonate with feeding intolerance, failure to thrive, vomiting, lethargy, and maple-syrup odor to urine and cerumen. If untreated, it progresses to irreversible mental retardation, hyperactivity, failure to thrive, seizures, coma, cerebral edema, and possibly death.

MSUD is a panethnic condition but is most prevalent in the Old Order Mennonite community in Lancaster, Pennsylvania with an incidence there of 1 in760 live births. The incidence of MSUD is approximately 1 in 185,000 live births in the general population.

Newborn screening includes the measurement of BCAA (Leu, Ile, and Val), which are elevated in MSUD. However, unaffected infants receiving total parenteral nutrition frequently have increased levels of BCAA, a situation that often triggers unnecessary follow-up investigations. Abnormal concentrations of allo-isoleucine (Allo-Ile) are pathognomonic for MSUD. The determination of Allo-Ile (second-tier testing) in the same newborn screening specimens that reveals elevated BCAA allows for positive identification of patients with MSUD and differentiation from BCAA elevations due to dietary artifacts, reducing the occurrence of false-positive newborn screening results.

Treatment of MSUD aims to normalize the concentration of BCAA by dietary restriction of these amino acids. BCAA are essential amino acids, which require frequent adjustment of the dietary treatment. Dietary monitoring is accomplished by regular determination of BCAA and Allo-Ile concentrations.

Reference Values

Allo-isoleucine: <2 nmol/mL

Leucine: 35-215 nmol/mL

Isoleucine: 13-130 nmol/mL

Valine: 51-325 nmol/mL
An interpretive report will also be provided.

Interpretation
Allo-isoleucine is nearly undetectable in individuals not affected by maple-syrup urine disease (MSUD). Accordingly, its presence is diagnostic for MSUD, and its absence is sufficient to rule-out MSUD.

Cautions
No significant cautionary statements

Supportive Data
In a blinded study containing specimens obtained from maple-syrup urine disease (MSUD) cases (n=16), non-MSUD patients treated with total parenteral nutrition (n=19), and healthy controls (n=541), this assay correctly identified all MSUD and non-MSUD cases.

Clinical Reference
1. Chace DH, Kalas TA, Naylor EW: Use of tandem mass spectrometry for multianalyte screening of dried blood specimens from newborns. Clin Chem. 2003 Nov;49(11):1797-1817. doi: 10.1373/clinchem.2003.022178
2. Simon E, Fingerhut R, Baumkotter J, Konstantopoulou V, Ratschmann R, Wendel U: Maple syrup urine disease: Favorable effect of early diagnosis by newborn screening on the neonatal course of the disease. J Inherit Metab Dis. 2006 Aug;29(4):532-537. doi: 10.1007/s10545-006-0315-y
3. Morton DH, Strauss KA, Robinson DL, Puffenberger EG, Kelley RI: Diagnosis and treatment of maple syrup disease: a study of 36 patients. Pediatrics. 2002 Jun;109(6):999-1008. doi: 10.1542/peds.109.6.999
4. Strauss KA, Puffenberger EG, Carson VJ: 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 December 16, 2020. Available at www.ncbi.nlm.nih.gov/books/NBK1319/

Performance

Method Description
This method quantifies valine (Val), allo-isoleucine (Allo-Ile), leucine (Ile), and leucine (Leu) using stable isotope-labeled internal standards (IS): d8-Val, d10-Allo-Ile, and d3-Leu. Branched-chain amino acids (BCAA) are extracted from a 3/16-inch dried blood spot (DBS) using a methanol:water (50:50) solution containing the IS. The filter plate containing the DBS and the IS are placed on an orbital shaker for 30 minutes at ambient temperature. The blood spot eluate is centrifuged into a 96-well round-bottom plate, dried under nitrogen, and reconstituted in aqueous mobile phase. BCAA are separated and detected by liquid chromatography-tandem mass spectrometry in positive selected reaction monitoring mode. Chromatography is performed using an Applied Biosystems AAA C18 (4.6x150 mm) column, with mobile phases 0.1% formic acid:0.01% heptofluorobutyric acid (HFBA):water and 0.1% formic acid:0.01% HFBA:acetonitrile. Total analysis time is 15 minutes including column re-equilibration.(Oglesbee D, Kramer K, Lacey J, et al: Second-tier test for quantification of alloisoleucine and branched-chain amino acids in dried blood spots to improve newborn screening of maple-syrup urine disease [MSUD]. Clin Chem. 2008 Mar;54(3):542-549. doi: 10.1373/clinchem.2007.098434; Stroek K, Boelen A, Bouva MJ, et al. Evaluation of 11 years of newborn screening for maple syrup urine disease in the Netherlands and a systematic review of the literature: Strategies for optimization. JIMD Rep. 2020 May 13;54(1):68-78. doi: 10.1002/jmd2.12124)

PDF Report
No

Specimen Retention Time
1 year

Performing Laboratory Location
Rochester

Fees & Codes

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 US Food and Drug Administration.

CPT Code Information
82136