Test Definition: ALLOI

Allo-isoleucine, Blood Spot

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)
-Card-Postmortem Screening (Filter Paper) (T525)
Container/Tube:
Preferred: Blood Spot Collection (Filter Paper)
Acceptable: Local newborn screening card, Whatman Protein Saver 903 filter paper, PerkinElmer 226 filter paper, Munktell filter paper, Postmortem 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 is a fingerstick. For detailed instructions, see How to Collect a Dried Blood Spot Sample.

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.

Specimen Minimum Volume

1 Blood spot

Reject Due To

Blood spot specimen that shows layering	Reject
Blood spot specimen that shows serum rings	Reject
Blood spot specimen that shows multiple applications	Reject
Blood spot specimen that shows insufficient specimen	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Whole blood	Ambient (preferred)	59 days	FILTER PAPER
	Refrigerated	59 days	FILTER PAPER
	Frozen	59 days	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 intellectual disability, hyperactivity, failure to thrive, seizures, coma, cerebral edema, and possibly death.

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 reveal 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: <4 nmol/mL
Leucine: 52-269 nmol/mL
Isoleucine: 22-167 nmol/mL
Valine: 84-414 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;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;29(4):532-537. doi:10.1007/s10545-006-0315-y
3. 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, 2025. Available at www.ncbi.nlm.nih.gov/books/NBK1319/
4. Frazier DM, Allgeier C, Homer 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. doi:10.1016/j.ymgme.2014.05.006
5. Blackburn PR, Gass JM, Vairo FPE, et al. Maple syrup urine disease: mechanisms and management. Appl Clin Genet. 2017;10:57-66. doi:10.2147/TACG.S125962

Performance

Method Description
Quantitative analysis of amino acids is performed by liquid chromatography tandem mass spectrometry (LC-MS/MS). Patient samples are combined with isotopically labeled internal standard. Following extraction, the filtrate is subjected to hydrophilic interaction liquid chromatography for the separation of isomers with MS/MS detection of the underivatized amino acids.(Unpublished Mayo method)

PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
2 to 4 days

Specimen Retention Time
1 year

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

Test Definition: ALLOI

Allo-isoleucine, Blood Spot

82136

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
ALLOI	Allo-isoleucine, BS	94571-7

Result ID	Test Result Name	Result LOINC® Value
27457	Allo-isoleucine	94572-5
27458	Leucine	47679-6
27459	Isoleucine	47671-3
27460	Valine	47799-2
27453	Interpretation	46743-1
27455	Reviewed By	18771-6